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SOME DIETARY FACTORS INFLUENCING CARDIAC RIGOR IN ALBINO RATS

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Hoet and Marks (1926) observed a rapid onset of rigor mortis in skeletal muscles of animals fed thyroid. McIntyre (1931) reported tachycardia in the denervated hearts of dogs following the feeding of desiccated thyroid.

McCarrison (1919) described cardiac atrophy caused by vitamin B deficiency. Patras and Templeton (1933) showed that restriction of dietary salts had a beneficial effect on the general condition, and prolonged the life of rats on a vitamin B deficient diet.

Bierfreund (1888) studied the effect of temperature on the development of rigor mortis in skeletal muscle. Using the hind legs of rabbits he observed that rigor at 4°C. began in one and one-half hours, while at 35°C. it began in two to five minutes. These observations were confirmed and extended by Morgenstern (1927).

Observations on the physical changes taking place in the heart following death of the animal have been made by several investigators, Brouardel (1889), Strassmann (1896), Fuchs (1900), MacWilliam (1901), Rothberger (1903), Joseph and Meltzer (1909), and Hans Meltzer (1927).

Since it has been shown that certain dietary factors and metabolic conditions of animals affect the function of the heart we thought these changes might be reflected in the survival period of the heart after sacrifice. Experiments were planned to determine if the influence of restricted salt intake, vitamin B deficiency, starvation, and desiccated thyroid ingestion could be measured by a study of rigor mortis in the excised hearts of albino rats.

METHOD. The animals used were selected from The University of Chicago and Loyola University Medical School colonies. Previous to the

experiments about one half of the animals were fed a stock diet consisting of Purina Fox Chow, with the addition, once per week, of beef hearts, bread and cabbage. The remaining animals received corn meal, powdered skimmed milk, bread and beef hearts daily.

The experimental diets (table 1) were all modifications of a standard diet. While the standard (diet 10) was known to be somewhat deficient it did serve to keep the animals in a good state of nutrition for the length of time that the experiments required.

Litter mates of the same sex and as near the same weight as practical were divided into groups and kept under comparable conditions with only the experimental variations. The desired physiological change was in some cases judged by the number of days the animals had been kept on their diet, while in other cases the weight curve was taken as the index. Immediately after the sacrifice, which was effected by a blow on

TABLE 1

	STANDARD	SALT DEFICIENT	VITAMIN B DEFICIENT	VITAMIN B AND SALT DEFICIENT	DESICCATED THYROID DIETS		
	Diet 10	Diet 11	Diet 12	Diet 13	Diet 14	Diet 15	Diet 16
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Casein	18	18	18	18	18	18	18
Starch	49	52	59	62	48.98	48.95	48.90
Butter	15	15	15	15	15	15	15
Yeast	15	15	5	5	15	15	15
Salt	3	0	3	0	3	3	3
Thyroid	0	0	0	0	0.02	0.05	0.10

the head, the thoracic cavity was opened, the heart removed and suspended in mammalian Ringer's solution thermostatically controlled. The temperature was kept at 30°C. except when the influence of various temperatures was being studied. The apex of the isolated heart was fastened to the end of a bent metal rod which could be lowered into the constant temperature bath. The base of the heart was hooked on a bent pin and connected to a heart lever with a silk string. This procedure required from 40 to 100 seconds. The activity of the heart was then recorded on a smoked drum. The duration of the different phases in the survival of the heart was measured in minutes.

The tracings obtained revealed three natural periods. Period I began with the stunning of the animal and ended when a contraction of the heart was followed by at least one minute of quiet. Period II, the quiet phase, had its beginning with the close of the active period and ended

with the beginning of rigor. Period III, the rigor phase, had its beginning at the close of the quiet period and ended when no further shortening of the heart was measurable. The sum of these natural divisions was considered a 4th period. It had its beginning with the stunning of the animal and ended with the completion of rigor. The mechanical difficulties encountered by the heart overcoming the inertia of the apparatus as it went into rigor made the establishment of the division between the quiet period and the period of rigor the least accurate.

TABLE 2

EXPERIMENT	GROUP	SUBGROUP	NUMBER OF RATS	DIET NUMBER	LOSS OF WEIGHT	DURATION OF PERIODS				APPROXIMATE RATIO			
						Active P I	Quiet P II	Rigor P III	P IV	P I	P II	P III	P IV
					per cent	*minutes*	*minutes*	*minutes*	*minutes*				
1	1		55	10		6.6	15.5	20.4	42.5	1	2	3	6
	2	A	19	12	15 or less	4.8	32.4	18.1	55.3	1	7	4	12
		B	24	12	16–30	4.9	30.3	23.3	58.5	1	6	5	12
		C	14	12	31–45	3.7	32.7	24.9	61.3	1	9	7	16
2	1		16	10		7.2	17.6	20.0	44.8	1	2	3	6
	2		16	11		6.8	20.2	18.0	45.0	1	3	3	7
	3		16	13	20 or less	5.6	26.0	18.8	50.4	1	5	3	9
	4	A	9	12	30 or less	5.7	36.0	20.7	62.4	1	6	4	11
		B	7	12	Over 30	5.2	44.4	21.7	71.3	1	9	4	14
3	1		14	10		6.1	15.8	21.2	43.1	1	3	3	7
	2		14		17–47	9.4	22.5	22.4	54.3	1	2	2	6
4	1		22	10		8.4	17.1	18.6	44.1	1	2	2	5
	2		22	14		6.5	16.3	16.0	38.8	1	2	2	6
	3		22	15		6.3	10.0	17.0	33.3	1	2	3	5
	4		22	16		4.3	4.5	14.7	23.5	1	1	3	5

Vitamin B deficiency. One hundred and twelve rats, varying in age from 33 to 68 days, (table 2, exper. 1) were divided into two groups. In group 1 there were 55 rats kept on the standard diet and in group 2 there were 57 rats which received the vitamin B deficient diet. These animals were sacrificed 35 to 75 days after the beginning of the experiment. Some of the animals on the vitamin B deficient diet lost weight much more rapidly than others, necessitating a division of group 2 into subgroups for comparison. Subgroup A lost 15 per cent or less of their maximum weight. Subgroup B lost 16 to 30 per cent of their weight, and subgroup C showed an emaciation of 31 to 45 per cent.

A comparison of the time required to complete the three phases of developing rigor in groups 1 and 2 showed the tendency of vitamin B deficiency to decrease the active period and increase the quiet and rigor periods as emaciation became more pronounced.

The normal time ratio of 1 to 6 existing between period I and period IV of the control group was raised to a ratio of 1 to 16 in advanced emaciation. This change in ratio was produced mainly by a decrease in the duration of the active period and an extension of the quiet period, supplemented by some increase in the period of rigor when the loss of weight exceeded 15 per cent.

Dietary salts and vitamin B deficiency. Sixty-four rats were divided into four groups (table 2, exper. 2) for the purpose of studying the influence of dietary salts on cardiac rigor. Group 1 was kept on diet 10 to establish controls. Group 2 received diet 11 which differed from the standard diet only in the substitution of starch for dietary salts. Group 3 was fed diet 13 which was deficient in both vitamin B and salts. Group 4 received diet 12 which was deficient in vitamin B. The animals varying in age from 47 to 66 days were kept on the above diets 40 to 45 days before sacrifice.

The simple restriction of salts (group 2) caused a slight reduction in the active period. A small increase in the duration of the quiet period was compensated for by a decrease in the rigor period making period IV practically the same as that of the controls.

The animals receiving the vitamin B and salt deficient diet (group 3) lost less than 20 per cent of their weight before sacrifice. The active period was decreased while the quiet period was increased. The period of rigor was increased relative to the group receiving the salt deficient diet but decreased in comparison to the standard group. Period IV was increased appreciably mainly by the increase in the quiet period.

Where only vitamin B was restricted (group 4) the animals were divided into two subgroups. Subgroup A included 9 rats which were emaciated 30 per cent or less before sacrifice. Subgroup B included 7 rats which had lost more than 30 per cent of their weight before sacrifice. Where emaciation was less than 30 per cent (subgroup A) the active periods were not significantly affected, but further emaciation of the animals on vitamin B deficiency (subgroup B) showed a decrease in the duration of the active period. The quiet period was lengthened in both subgroups, increasing with the loss of weight. In the period of rigor the influence of the loss of weight was not so conspicuous. However, some extension of this period was observed relative to the degree of emaciation. Period IV was increased relative to the degree of loss of weight, largely by extension of the quiet period and slightly by extension of the period of rigor in case of advanced emaciation.

The relations of the periods to one another were practically unchanged when only the dietary salts were restricted, but in the case of advanced emaciation in vitamin B deficiency the relation between the active period and period IV was raised from a normal of 1 to 6 ratio to a ratio of 1 to 14.

Starvation. Since animals lose their appetite for vitamin B deficient diets the degree of emaciation is undoubtedly influenced to some extent by general starvation. To control this aspect 28 rats between the ages of 53 to 90 days (table 2, exper. 3) were kept on the standard diet for 30 days and then divided into two groups. Group 1 was continued on the standard diet for controls. Group 2 was subjected to starvation for 3 to 6 days before sacrifice. In this time the animals lost 17 to 47 per cent of their body weight.

All of the periods were increased by starvation. This effect was similar to that produced by vitamin B deficiency except that the period of activity was increased in starvation which was just opposite to that observed in vitamin B deficiency. This experiment, of course, considers only acute starvation. It is possible that if the animals were slowly starved by simply restricting the food intake to a time comparable to the vitamin B deficient experiments, the active periods might be affected in the same direction.

In the dietary deficiencies (starvation and vitamin B deficiency) the time required for the completion of rigor was lengthened roughly in proportion to the degree of emaciation. This increase may be more closely related to the change in metabolism than to the degree of emaciation as suggested.

Thyroid. The feeding of desiccated thyroid is known to cause a loss in weight and at the same time to increase the metabolic rate. To study this effect on cardiac rigor 88 rats between 32 and 68 days of age (table 2, exper. 4) were divided into four groups. One group received the standard diet. A second was fed diet 14 (table 1) which contained 0.02 per cent thyroid. The third group received 0.05 per cent of thyroid (diet 15) and the fourth 0.1 per cent of thyroid (diet 16). The animals were sacrificed after being on their respective diets between 21 and 60 days.

Animals receiving 0.02 per cent thyroid for only 21 days showed an onset of cardiac rigor after death which was practically the same as that observed in the controls. After 40 days, however, all of the periods were shortened. The influence of 0.05 per cent of thyroid in the diet had an earlier and more profound effect in the shortening of all the periods studied. The effect of 0.1 per cent thyroid was exerted to a measurable extent in 21 days and became more profound as the duration of the experiment was extended.

The influence of thyroid feeding is summed up in experiment 4 of table 2. Increasing the intake of thyroid caused a decrease in the dura-

tion of all the periods leading to the completion of rigor. Thyroid feeding had very little effect on the ratio existing between the periods. This is comparable to the results obtained by starvation. However, the results here were obtained by shortening all of the periods while starvation lengthened all of the periods.

Temperature. The influence of temperature on cardiac rigor was determined on 71 rats varying in age from 38 to 90 days in order to secure data to which the various effects obtained in the previous experiments might be referred. The animals were kept on the standard diet for about 60 days. The range of temperature studied varied from 20° to 40°C. Since it was impossible to obtain litters large enough to have one littermate representative at each temperature studied, the animals were distributed in such a way as to have litter-mates overlapping at as many points

TABLE 3

TEMPERATURE	DURATION OF PERIODS				APPROXIMATE RATIO			
	Active P I	Quiet P II	Rigor P III	P IV	P I	P II	P III	P IV
°C.	*minutes*	*minutes*	*minutes*	*minutes*				
20	15.6	51.3	36.0	102.9	1	3	2	7
24	14.4	36.5	23.0	73.9	1	3	2	5
26	12.6	28.6	19.5	60.7	1	2	2	5
28	8.6	25.5	21.4	55.5	1	3	3	6
29	7.1	20.6	15.8	43.5	1	3	2	6
30	6.6	15.4	20.4	42.4	1	2	3	6
31	7.5	21.6	14.0	43.1	1	3	2	6
32	5.7	12.9	20.6	39.2	1	2	3	7
34	4.3	15.5	11.8	31.6	1	3	3	7
36	4.3	11.8	12.1	28.2	1	3	3	7
38	3.4	6.2	12.5	22.1	1	2	3	6
40	3.2	6.9	8.7	18.8	1	2	3	6

as possible. In addition, the hearts of 55 rats, between the ages of 33 to 68 days, kept on the standard diet from 35 to 75 days were isolated and studied at 30°C. Since this temperature was used in all the experimental work, it was considered most important to accurately establish this point.

Changes in temperature did not materially alter the ratio between the active period and the other phases (table 3). In general the active period became progressively shorter as the temperature was increased. Proportionally, however, the other periods were also decreased.

Although there was more irregularity between the temperature of 26° to 32°C., period IV, which measures the total time from the stunning of the animal to the completion of rigor, gives a fairly smooth curve. At 20°C. the rigor was not complete until 102.9 minutes after the animal was stunned, while at 40°C. the completion of rigor occurred in 18.8 minutes.

To the results obtained by varying the temperature other conditions can be referred. It is possible to express in degrees of temperature the extent to which the various physiological changes already studied altered the phases in the survival time of the heart after death of the animal.

The control animals for the vitamin B deficient experiment (table 2, exper. 1) showed cardiac rigor complete in 42.5 minutes. This is comparable to the figure obtained at 30°C. (table 3) in the study of the influence of temperature. The vitamin B deficient animals emaciated 15 per cent or less showed rigor complete in 55.3 minutes, which when compared to data in the temperature table indicates that this degree of vitamin B deficiency is equivalent to lowering the temperature on a normal heart by approximately 2°C. As emaciation progressed the completion of rigor was delayed so that in those animals emaciated 16 to 30 per cent rigor was complete in 58.5 minutes which is equivalent to lowering the temperature by about 3°C. on normal hearts. Emaciation more than 30 per cent further increased the time involved in the completion of rigor to 61.3 minutes which is equivalent to lowering the temperature on the normal heart by approximately 4°C.

Simply restricting the salt in the standard diet had practically no effect on the time required for the completion of rigor (table 2, exper. 2). The control animals for this experiment showed cardiac rigor complete in 44.8 minutes. When vitamin B deficient animals were also subjected to salt restriction rigor was completed in 50.4 minutes, which is equivalent to lowering the temperature on their controls only about 1°C.

The controls for the starvation experiment (table 2, exper. 3) were practically the same as the standards used at 30°C. to establish the temperature data (table 3). The animals subjected to starvation showed the completion of rigor prolonged to 54.3 minutes, which is equivalent to lowering the temperature on their controls approximately 2°C.

The controls for the thyroid fed animals (table 2, exper. 4) went into rigor nearly two minutes later than the animals used at 30°C. to establish the temperature curve (table 3). The feeding of 0.02 per cent of thyroid hastened the completion of rigor to 38.22 minutes, which is equivalent to raising the temperature on their controls by approximately 3°C. Feeding 0.05 per cent thyroid is equivalent to raising the temperature of the controls about 4°C., while feeding of 0.1 per cent thyroid is equivalent to raising the temperature by approximately 8°C.

SUMMARY

1. The duration of rhythmic cardiac contractions (active period) following extirpation was increased by starvation, and decreased by vitamin B deficiency, experimental hyperthyroidism and to a slight extent by salt deficiency.

2. The time between the close of spontaneous activity and the onset of rigor (quiet period) was increased by vitamin B deficiency, starvation, and to a less extent by salt deficiency. This period was decreased by experimental hyperthyroidism.

3. The period during which rigor developed (rigor period) was increased by vitamin B deficiency when the animals had lost 30 per cent or more of their weight and by starvation. A decrease in the duration of this period was observed in vitamin B deficiency when the loss in weight of the animals before sacrifice did not exceed 20 per cent. Salt deficiency and experimental hyperthyroidism also caused a decrease in this period.

4. The ratio of the time of functional activity (active period) to the total survival time (period IV) was increased by vitamin B deficiency, but practically not changed by salt deficiency, starvation or experimental hyperthyroidism. When salt deficiency was associated with vitamin B deficiency the influence was to decrease the effect of the vitamin deficiency.

5. The effects of vitamin B deficiency, salt deficiency, starvation, and experimental hyperthyroidism on the survival period were compared to the influence of temperature, and can be expressed quantitatively in terms of temperature.

We are indebted to Dr. A. J. Carlson under whose direction this work was conducted.

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THE DISTRIBUTION OF GLUCOSE BETWEEN BLOOD CELLS AND SERUM

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The free permeability of the human red blood cell membrane to glucose in concentrations up to 700 mgm. per cent, both *in vivo* and *in vitro*, has been generally recognized since Somogyi, in 1929, published the details of an adequate and accurate method of determining the blood glucose. Somogyi, Power and Ege (4, 11, 12) have found the ratio $\frac{\text{Glucose of cells}}{\text{Glucose of plasma}}$ to vary between 0.7 and 0.85. MacKay (9) has ascertained the ratio $\frac{\text{Glucose in water of cells}}{\text{Glucose in water of plasma}}$ by determining in every case the water content of cells and plasma. He found the ratio to vary between 0.96 and 1.01. Slightly different findings have been reported by Folin and Andreen-Svedberg (1, 6), who reported $\frac{\text{Glucose of cells}}{\text{Glucose of plasma}}$ ratios of 0.6 in blood of normal persons. The method employed, however, invalidates this difference (see Somogyi, 12b), and when proper corrections are made, their experiments are found to check quite well with those of other investigators.

A more fundamental challenge to the current concept has been sounded recently by Olmsted (10). He concludes that the human red blood cell contains little or no glucose, and that the large amounts usually found are due to damage to the cell membrane and the subsequent free penetration of glucose from the plasma. To minimize the trauma he withdrew venous blood under oil, centrifuged a portion of it, and removed the plasma before coagulation could occur, within 4 minutes of withdrawal from the body. Determining the glucose content of the plasma so separated, that of the whole blood, and the cell volume, he was able to calculate the glucose content of the cells. His data offer a striking contradiction to the prevailing opinion, for in typical experiments he found the corpuscular glucose to change from 10 and 5 mgm. per cent to 30 and 33 respectively on the addition of oxalate, while the plasma glucose content conversely fell from 108 and 77 mgm. per cent to 95 and 56. In other

experiments he determined the plasma and whole blood glucose of oxalated blood immediately on withdrawal from the vein, and at intervals for one and a half hours. In these experiments he found at first only a small quantity of glucose in the cells (as little as 30 mgm. per cent), and this amount progressively increased, while that in the plasma correspondingly decreased, in one experiment falling from 140 mgm. per cent to 72, the ratio of $\frac{\text{Glucose of cells}}{\text{Glucose of plasma}}$ changing from 0.21 to 1.01. He ascribes the increase in the quantity of glucose in the cells to damage of the cell membrane by the potassium oxalate.

We have repeated Olmsted's experiments without, however, being able to duplicate his results. In all cases the $\frac{\text{Glucose of cells}}{\text{Glucose of plasma}}$ ratio lay between 0.58 and 0.86, in no case was there a significant difference between the amounts of glucose in the plasmas of oxalated and unoxalated blood, and there was no significant change with time in the glucose content of the cells or plasma of oxalated blood, except for that due to glycolysis. The ratio varied with time from an average of 0.71 to one of 0.73 (tables 1 and 2).

EXPERIMENTAL PROCEDURES. In all cases venous blood was used. It was secured from normal subjects, from patients with diabetes mellitus, and in two instances, from patients with polycythemia. In the experiments reported in table 1 the blood was withdrawn anerobically, some of it centrifuged immediately without exposure to air and without the addition of an anti-coagulant, and some centrifuged after the addition of potassium oxalate. In either case the plasma was separated within 5 minutes of withdrawal of the blood from the body, and added to the Somogyi zinc precipitant for the quantitative determination of glucose. Benedict's reagent was used (2, 12a). In experiment A the whole blood glucose and the cell volume were also determined, and the cellular glucose calculated. In experiment D the blood was chilled. In the experiments reported in table 2, the blood was oxalated and the plasma separated within 10 minutes of withdrawal from the body, and from another portion again after one and a half to two hours. In both specimens plasma and whole blood glucose were determined and the cell volume was measured in 5 cm. hematocrit tubes according to the method of Eisenman (5). The results with this method are accurate within one volume per cent. The glucose content of the cells was calculated from the available data without correction of the cell volume for the addition of oxalate. In the one and a half hour interval between determinations the blood was kept at room temperature.

Olmsted's high values for the glucose of plasma are not readily explicable; they are quite at variance with the data already referred to and

those presented in table 1. There is a feasible method of estimating the glucose content of the plasma *in vivo* with absolutely no disturbance of the red cell membrane or of the ratio of glucose in cells and plasma: determining the quantity of glucose present in transudates. Unless the capil-

TABLE 1

Glucose in plasma removed from blood without the addition of an anticoagulant, and following addition of potassium oxalate

EXPERIMENT	PLASMA GLUCOSE (NO ANTICOAGULANT)	PLASMA GLUCOSE (OXALATED BLOOD)	WHOLE BLOOD GLUCOSE	CELL VOLUME	CELL GLUCOSE
	mgm. per cent	*mgm. per cent*	*mgm. per cent*		
A	82	84	73	42	58
B	112	100	100		
C	94	93			
D*	101	105			

* Chilled.

TABLE 2

Glucose in whole blood, plasma, and red blood cells following the addition of potassium oxalate to whole blood

EXPERIMENT	10 MINUTES					1½ HOURS			
	Cell volume	Mgm. glucose per 100 cc.				Mgm. glucose per 100 cc.			
		Whole blood	Plasma	Cells	$\frac{\text{Cells}}{\text{Plasma}}$	Whole blood	Plasma	Cells	$\frac{\text{Cells}}{\text{Plasma}}$
	per cent								
1	49.4	210	250	169	0.67	197	232	161	0.69
2	59.2	105	128	90	0.71	95	114	82	0.72
3	37.0	101	110	88	0.80	94	101	82	0.81
4	36.0	86	101	59	0.58	73	82	49	0.60
5	38.9	185	197	166	0.84	183	193	167	0.86
6	51.2	269	317	222	0.70	243	285	202	0.71
7	43.1	88	101	71	0.72	77	87	70	0.83
8	41.0	89	100	73	0.73	81	87	72	0.83
9	40.0	186	209	166	0.79	164	192	133	0.70
10	36.0	88	99	70	0.70	74	83	58	0.70
11	31.0	77	89	51	0.58	72	81	51	0.63
12	39.2	73	84	56	0.67		79		
Average (exper. 12 excluded).....					0.71				0.73

lary wall has properties of which we are unaware, the amount of glucose present in transudates should be approximately equal to that present in plasma. Several investigators (3, 7) have found this to be true, even when the glucose of the plasma is determined from oxalated blood, and not after such careful precautions as those of Olmsted.

Olmsted's ratio of 1.01 for $\frac{\text{Glucose in cells}}{\text{Glucose in plasma}}$ of oxalated blood after one and a half hours also is rather unconvincing. Assuming that potassium oxalate damages the membrane of the cell to allow the free diffusion of glucose into it, one is then under the necessity of explaining why the concentration of glucose in the water of the cells should be so much greater than its concentration in the water of the plasma.[1] Available evidence indicates that glucose within the cell is in a freely diffusible form, and there is no other evidence to suggest that the red cell membrane under certain conditions has a one way permeability to glucose (8).

Until, therefore, more convincing evidence to the contrary is adduced, we must accept as proved the free permeability of the human red cell membrane to glucose, and the equal distribution of glucose in the water of cells and plasma.

Since this article was written Neuwirth has published a paper establishing substantially the same facts (This JOURNAL **117:** 335, Oct. 1936).

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[1] The cell contains only about 72 per cent water by volume, whereas the plasma contains about 93 per cent. If glucose is equally distributed in the water of cells and plasma the ratio should approach 0.7, and not 1.0.

ALTERATIONS OF ALVEOLAR CO_2 IN MAN ACCOMPANYING POSTURAL CHANGE

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There are various explanations of the drop in alveolar CO_2 in man upon assuming the erect posture. Liljestrand and Wollin (1) believe that the increased vital capacity may be responsible. Higgins (2) states that the more relaxed the position, as in reclining, the higher is the alveolar CO_2, and suggest that perhaps this is due to a vaso-dilatation accompanying relaxation. Turner (3) corroborates Higgins' observations in general but says the lowered CO_2 when sitting and standing may be related to the disadvantage to the circulation of an erect posture, and that the diminished CO_2 may be due directly to the increased ventilation of a standing position: a hyperpnea resulting from the now inadequate cerebral circulation.

I. Several other possibilities of explaining this phenomenon exist. The production of acid metabolites as lactic acid, associated with the increased metabolism of the contracting muscles on standing, may diminish the blood bicarbonate or pH, and therefore diminish alveolar CO_2. To analyze this factor, the blood pH and alveolar CO_2 of three subjects were determined while lying and standing.

METHOD. The Haldane-Priestley (4) method for collecting alveolar air was used, with the exception that only the expiratory sample was taken, since this agrees with the CO_2 tension of arterial blood (Bock and Field, 5; Dautrebande, 6). The subjects were students between the ages of twenty and thirty years, and experiments were run after meals, rather than in the fasting "basal" condition, since we have unpublished evidence that the alveolar CO_2 tension exhibits greater spontaneous variations during hunger. The subjects were always requested to relax as completely as possible; talking, smoking, and unnecessary movements were prohibited, and the bladder was previously emptied. Care was taken not to use a subject when drowsy. Samples were taken at five- or ten-minute intervals, in order to compensate for the normal physiological variations. Results are reported in per cent of one atmospheric pressure. The Shock-Hastings micro method (7) for blood pH was employed. A blood sample

was drawn from an antecubital vein after the subject had been lying for a period varying from thirty to sixty minutes. He then stood up from one to ten minutes after this venipuncture. A second blood sample was taken four to six minutes after standing.

These experiments all showed a consistent rise of 0.03 pH to 0.06 pH upon standing: a value approximating that which could be expected from study of the carbon dioxide absorption curve of human blood when the alveolar CO_2 tension is reduced as from 6.1 per cent to 5.4 per cent. The second blood sample was taken from four to six minutes after standing because the alveolar CO_2 has reached approximately its new level within one minute after change of posture, but more time than this must be required for the blood to attain equilibrium. Blood samples taken after standing for more than twenty minutes showed a diminution of pH toward the lying value, due perhaps to compensatory changes. This result proves that the drop in alveolar CO_2 pressure upon standing, usually between 0.5 and 1.0 per cent, cannot be due to an acidemia but is secondary to an over-ventilation, which thereby produces an alkalemia. The increased ventilation on standing noticed by Liljestrand and Wollin (8) may then be a true over-ventilation above that required to excrete the increased CO_2. It is necessary, therefore, to seek some factor occurring upon standing, which may be capable of over-stimulating the respiratory center.

II. The increased body temperature on standing (Kleitman and Doktorsky, 9), however, would seem capable of explaining a concomitant over-ventilation, since alveolar CO_2 of man is lowered upon increasing the body temperature by the external application of heat (Haldane, 10). This usual lowering of alveolar CO_2 is apparently due to respiratory stimulation by heat, either by reflex or direct action upon the center.

In order to investigate the possibility that the temperature rise on standing might be responsible for the lowered alveolar CO_2, we used the following procedure: For the determination of body temperature, the rectal piece of a Leeds-Northrup electrical recording thermometer was employed, since determinations of oral temperatures with a clinical thermometer were found unsatisfactory. Two subjects, previously accustomed to its use, put the rectal piece in place and assumed the supine position. As soon as the temperature had reached a level, alveolar air samples were taken at ten-minute intervals. At the end of the hour they stood up and the next sample was taken three to five minutes later. Owing probably to the latent heat of the body, no change in body temperature took place in this brief interval, and yet figure 1 shows that the new level of alveolar CO_2 had already dropped to a value which then remained relatively constant, even though the temperature later began to rise. This rapid assumption of the new CO_2 level within a few minutes after change of posture, long before any change of body temperature occurs, indicates

that some factor other than temperature must be responsible. The relatively level alveolar CO_2 values following the immediate drop, in spite of the later slow temperature increase, would in fact indicate that the part played by such a change in body temperature is negligible in comparison to other factors. Comparable results were obtained by starting with the subject standing for an hour and then lying down. Hence, we must conclude that the fall in alveolar CO_2 caused by standing is not due to the rise in body temperature stimulating the respiratory center.

III. We must next consider the suggestion of Turner (3) that the diminution of alveolar CO_2 in the erect position may be due to a hyperpnea caused by a relative cerebral anemia stimulating the respiratory center. I attempted to analyze the probability of Turner's suggestion, by determining the subject's alveolar air at five-minute intervals while lying, then standing erect, and then while standing bent over forward with his hands on his knees so that the head was practically level with the heart, in order to facilitate cerebral circulation. The alveolar CO_2 in the latter position

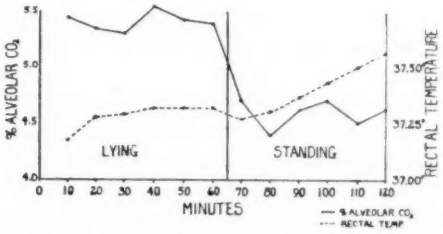

Fig. 1. The lack of correlation between the drop in alveolar CO_2 upon standing and the rise in rectal temperature.

(c. 5.8 per cent) is consistently practically the same as when lying (c. 5.7 per cent), and much higher than when standing erect (c. 5.2 per cent). This result would indicate that a diminished circulation to the brain or a diminution in the blood pressure in the carotid sinus, probably plays a part in the increased ventilation and lowered alveolar CO_2 of the standing position. However, if the increased hydrostatic pressure of the blood on assuming an erect posture produces such an inadequate cerebral circulation, then one might anticipate that a similar, but lesser, change would be produced upon sitting. Nevertheless, there is no appreciable difference in the alveolar CO_2 of trained subjects lying, or sitting in a comfortable chair in which they can relax completely. Thus, in spite of the fact that the upper portion of the body is erect, there is apparently no indication of cerebral anemia as would be evidenced by a drop in alveolar CO_2. For this reason, I do not consider that the idea of an ischemia of the brain

can be offered as the sole explanation of the over-ventilation and diminished alveolar CO_2 of the erect position, but believe that some other factor may be concerned. Nevertheless, when a subject is securely strapped to a tilting table and his position is passively changed from horizontal to vertical without necessity of any muscular contraction, his alveolar CO_2 shows a distinct drop, although usually less than when actively standing.

IV. The increase in vital capacity upon standing likewise must be considered as a possible explanation of the diminished CO_2 pressure upon rising, as suggested by Liljestrand and Wollin (1). However, since the alveolar CO_2 of a subject is practically the same when lying, or sitting in a comfortable chair, in spite of the fact that the vital capacity is greater sitting than lying, indicates that the change in vital capacity does not necessarily influence the respiratory center. When the vital capacity has been diminished by production of a unilateral pneumothorax, I have found that alveolar CO_2 falls in direct proportion to the diminution of vital capacity: a fact which corresponds to our unpublished observations indicating that among individuals, a large vital capacity per square meter of body surface area seems to be associated with a high alveolar CO_2 level. Thus, these findings would not lead us to expect that the enlarged vital capacity of the standing position would produce a drop in alveolar CO_2, but rather the reverse.

V. Finally, since the diminished alveolar CO_2 of standing does not readily appear to be due to acidemia, increased temperature, or enlarged vital capacity, and perhaps only in part to cerebral ischemia, we are forced to look still further for an explanation. The fact noticed by Higgins (2), that a high alveolar CO_2 seems to be associated with muscular relaxation, reminds us of the work of Harrison et al. (11) who showed that apparently proprioceptors are capable of stimulating respiration. Perhaps on standing then, impulses from those sensory endings in the tonically contracting muscles impinge upon the respiratory center and either increase its sensitivity to CO_2, or, more simply, actually stimulate it, just as other types of strong sensory stimuli will affect respiration, such as pain or cold. This increased stimulation of the respiratory center would, therefore, produce an enlarged ventilation with consequent diminution of alveolar CO_2 and resultant alkalemia.

In order to investigate this question, alveolar air samples were obtained in the usual way, on ten subjects lying, sitting and standing, an hour each. Complete relaxation was attempted. The sitting was done in an easy chair with the back slightly inclined so that no muscular contraction was necessary to maintain the position. It was soon found that some subjects, capable of remaining perfectly immobile, showed no marked difference in average alveolar CO_2 on lying and sitting. Others, who were obviously more restless, had a somewhat lower alveolar CO_2 when sitting, although not nearly so low as when standing.

The possibility that this restlessness and lack of relaxation in the sitting posture might prove to be an important factor in explaining the difference among various subjects, suggested that we have subjects sit on a stool necessitating muscular contraction to maintain their position. This usually produces a lower alveolar CO_2 level than when in a chair. Thus we are inclined to associate the diminution of alveolar CO_2 with contraction of the muscles—both greatest upon standing.

Discussion. The observation that alveolar CO_2 of a subject standing bent over is as high as when lying, strongly indicates that Turner's (3) explanation of the drop in alveolar CO_2 on standing as due to cerebral ischemia is probably correct. However, it is quite possible that this stimulation of respiration may be due to a drop in pressure in the carotid sinus, as well as an ischemia of the respiratory center. Still, the fact that the alveolar CO_2 when sitting completely relaxed approximates the value when lying, is difficult to explain, in view of the fact that the upper portion of the body is erect, and therefore should produce a similar cerebral ischemia. This discrepancy, together with the observation that a relaxed sitting posture produces a higher alveolar CO_2 than sitting requiring muscular contraction, necessitates the suggestion that proprioceptive stimulation of respiration may be a complicating factor. The above results therefore correspond to Higgins' (2) observation that the more relaxed the position, the higher is the alveolar CO_2.

If then this drop in alveolar CO_2 is due largely to cephalic ischemia, it it difficult to understand why compensatory circulatory adjustments do not occur, rather than permit a cerebral anemia to cause such over-ventilation as to produce a considerable disturbance of blood pH. Perhaps this is merely another example of the fact that physiologic compensation is rarely perfect.

The fact that the alveolar CO_2 tension is so dependent upon such factors as posture, indicates that alveolar CO_2 is not a physiologic constant, as originally believed by Haldane (4). Rather, it is a variable: the resultant of all factors affecting the respiratory center. Consequently, we cannot speak of an individual's alveolar CO_2 value unless we specify definitely the conditions under which it was taken.

SUMMARY

The diminished alveolar CO_2 upon standing is explained as being due to pulmonary over-ventilation with resulting alkalemia. This respiratory stimulation is not due to the rise in body temperature but is believed to be caused by stimulation of respiration due to cephalic ischemia. Proprioceptive stimulation may also be concerned. These observations are used to question the idea of alveolar CO_2 being a physiological constant.

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ALTERATION OF THE ACTIONS OF VARIOUS RESPIRATORY MODIFIERS BY LOCAL COOLING OF THE FLOOR OF THE FOURTH VENTRICLE[1,2]

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In a previous paper one of us (Nicholson, 1936) described the effects of local cooling of the floor of the fourth ventricle on respiration. Since it was found that local cooling of the floor of the fourth ventricle in the region of the calamus scriptorius markedly altered normal breathing, it was felt that a study of the effects of such cooling upon respiration modified by various experimental procedures might throw light, not only upon the actions of these various respiratory modifiers, but also upon the mode of functioning of the central respiratory mechanism itself. Our experiments have been performed upon dogs 18 to 22 kgm. in weight anesthetized with morphine and urethane.

First, we have studied the relationship of the vagus nerves to respiration as modified by local cooling of the posterior portion of the floor of the ventricle. This local cooling was accomplished by the use of the applicator described in our previous paper (Nicholson, 1936). Ordinarily faradic stimulation of the central end of a sectioned or blocked vagus nerve, if the current is strong, will completely inhibit respiration, the inhibition occurring with the chest in the expiratory position; if the current is of moderate strength there usually results an acceleration of respiration with a decrease in amplitude. However, stimulation of the central end of the vagus is much less effective in modifying respiration during local cooling of the posterior part of the floor of the fourth ventricle. During such cooling it is almost impossible to completely arrest respiration. A strength of current which would normally do this may, during cooling, be entirely without effect, may cause a slight slowing or a slight acceleration. Figure 1A, B, C, D, E and F illustrates this. The respiratory record is a spirometer tracing, upstroke representing inspiration. The numbers below the record indicate the position of the secondary coil in centimeters, increasing numbers representing greater approximation of the coils. It is seen in records 1 A and B that under normal conditions

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stimulation of the central end of the vagus with current 7 produced a marked slowing of respiration while current 8 caused complete arrest of respiration for the duration of the stimulus, about 22 seconds. However, stimuli of the same strengths applied to the vagus during local cooling of the region of the calamus scriptorius (1 C and D) caused only a slight acceleration of respiration, and a considerable increase in the strength of the current (1 E) served only to accentuate this acceleration with no sign of inhibition. After return of the calamus region to body temperature (1 F), current strength 8 was sufficient to cause complete inhibition of respiration.

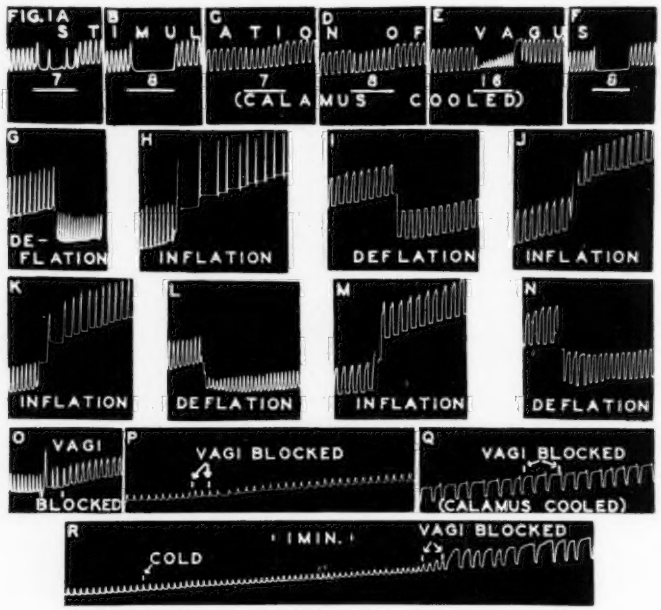

Fig. 1

We have also studied the effect of cooling upon two more nearly normal vagal responses, namely, the respiratory effects resulting from inflation and deflation of the lungs. This inflation and deflation were accomplished by increasing the weights of a mercury buoyed spirometer and of its counterweight respectively. Ordinarily, such pulmonary inflation while increasing the amplitude of respiration produces a marked slowing with an increase in the duration of the expiratory pause. Pulmonary deflation on the other hand ordinarily produces a decrease in amplitude and increase in rate with a shortening of the expiratory pause. Cooling the calamus region, if sufficiently marked, was found to greatly depress or

completely abolish these two reflex responses. In figures 1 G to J inclusive we see this effect of cooling upon the respiratory responses to inflation and deflation of the lungs. In figure 1 G a given negative pressure increased the respiratory rate from 19 to 33 per minute, an increase of about 74 per cent. In figure 1 H the same degree of positive pressure decreased the respiratory rate from 21 to 7 per minute, about 67 per cent. In figures 1 I and J the same degrees of negative and positive pressure *during cooling* were entirely without effect, the respiratory rate remaining constant at 12 per minute. Repetition of these procedures after return of the calamus region to body temperature produced the original effects. It was occasionally found, however, that a degree of cooling sufficient to diminish markedly the response to inflation of the lungs might be entirely without effect upon the response to deflation. In fact in a few cases it was found that deflation of the lungs caused a more marked acceleration of respiration during cooling of the calamus region than when that region was at body temperature. An example of this differential effect of cooling is provided by figure 1 K to N inclusive. In figure 1 K a given inflation caused a decrease in respiratory rate from 21 to 9 per minute, about 57 per cent, while deflation, in figure 1 L increased the respiratory rate from 21 to 24 per minute, about 14 per cent. As seen in figure 1 M during cooling of the calamus region the same positive pressure as before now decreased the respiratory rate only from 12 to 11 per minute, or 8 per cent. However, during cooling, negative pressure, shown in figure 1 N was slightly more effective than before, increasing the respiratory rate from 13 to 16½ per minute, about 27 per cent.

The above observation on the differential action of cooling on the effects of pulmonary inflation and deflation suggests that different vagal fibers with different central connections are involved in the mediation of the two effects or that accelerating fibers outside the vagus trunk are in some way involved. We have previously shown (Nicholson, 1936) that the typical effect of cooling the calamus region is an alteration in the relative duration of the inspiratory and expiratory phases of respiration in favor of the former. If the cooling is severe there is a marked prolongation of inspiration, i.e., apneustic breathing. If the cooling is slight there is merely a decrease in the duration of the expiratory pause. Now deflation of the lungs causes a change in the relative durations of inspiration and expiration in the same direction as does cooling while inflation causes a change in the opposite direction, that is, a prolongation of the expiratory pause. It is, therefore, to be expected that with moderate cooling the effects of deflation will be less markedly diminished or possibly even enhanced.

Further light upon the influence of the vagus nerves on respiration and the modification of this influence by cooling is provided by studying the

effects of blocking the vagi under various conditions. The usual effects of blocking the vagi are a decrease in respiratory rate, an increase in amplitude and a decrease in the speed with which inspiration proceeds. This last effect is, we believe, worthy of special consideration. It was apparently first mentioned by Gad (1880) but seems to have been largely overlooked in recent discussions of the subject. Gad believed that this increase in the duration of the inspiratory phase was the most characteristic effect of vagotomy upon breathing and was a more constant finding than either the increase in depth or decrease in rate, a view with which we are inclined to agree. Although this decrease in the speed of inspiration becomes more marked as inspiration approaches its end it is frequently present throughout inspiration and occurs whether or not there is any increase in the depth of inspiration. This is shown in figure 1 O. In this observation blocking the vagi caused no change in respiratory amplitude. The total duration of the respiratory cycle was increased 1.6 second or 64 per cent. The duration of the inspiratory phase of the cycle was increased 1.26 second or 161 per cent. That is, 79 per cent of the decrease in respiratory rate was accounted for by a decrease in the speed with which inspiration proceeded.

These effects of blocking the vagi are however markedly modified by cooling the floor of the fourth ventricle. Figure 1 P shows the effect of blocking the vagi under normal conditions. In this case the vagal block resulted in a decrease in respiratory rate from 12 to 10 per minute and an increase in the amplitude of the tracing from 5 to 7 mm. In figure 1 Q, from the same animal, the vagi are blocked during severe cooling of the calamus region. It is seen that the cooling itself has caused a marked slowing of respiration with a great prolongation of inspiration—typical apneustic breathing. Blocking the vagi during such apneustic breathing is entirely without effect. Evidently this *severe* cooling of the obex region has removed all vagal influence upon breathing. If, however, the vagi are blocked during *slight* cooling of the calamus region a striking effect is observed. As we have previously pointed out slight cooling of this region results not in apneusis but rather in a decrease in the duration of the expiratory pause with a resultant acceleration of respiration. (This effect has been observed as a result of lowering the temperature of the applicator as little as 3.0°C.) The same effect may result from severe cooling of a region a short distance (8 to 10 mm.) anterior to the obex, probably due to transmission of the cooling effect diminished in intensity to the obex region. Now, in an animal in which such an acceleration of respiration with shortened expiratory pause has been produced either by slight cooling of the calamus region or more severe cooling of a more anterior region, if the vagus nerves are sectioned or cold blocked there immediately results a well developed apneustic type of breathing. An ex-

ample of this is shown in figure 1 R from the same experiment as figure 1 P and 1 Q. In this case cooling of a region about 10 mm. above the obex caused an increase in respiratory rate from about 12.5 to 14 per minute with a definite shortening of the expiratory pause and a slight increase in the duration of inspiration. Blocking the vagi then caused a marked slowing of respiration with typical apneusis. Removal of the blocks from the vagi reversed the effect perfectly.

Apneusis then may be produced either by *severe* cooling of the calamus region (such cooling involves removal of the vagal influence upon breathing) or by very slight cooling of the calamus region plus blocking or section of the vagi. Elimination of the impulses travelling up the vagus nerves to the central respiratory mechanism seems to be essential to the production of apneusis but is not of itself sufficient. Depression of some portion of the respiratory mechanism by cooling is also necessary. Exactly what part of the respiratory mechanism this is and exactly what is its normal function we do not know. It is inconceivable that the slight amount of cooling required would seriously affect conduction in a nerve fiber but it might very readily alter the rate of discharge of a nerve cell and possibly depress conduction across a synapse.

We have no intention of formulating a theory regarding the activity of the central respiratory mechanism. However, we would like to again call attention to a fact mentioned by various workers, viz., the resemblance between respiration and various rhythmic reflexes such as stepping. In 1922 Forbes in a review of spinal reflexes outlined a scheme of connections of the spinal neurones which might conceivably explain reciprocal innervation and intrinsic alternation of flexion and extension, the author emphasizing, however, that he did not claim for it "the least degree of probability in its present form." Starting from the phenomenon of Wedensky inhibition this hypothesis suggested that an increase in the frequency of the impulses arriving at some internuncial neurone above a certain critical frequency might bring about inhibition in that group of neurones and thus produce the turning point in the reflex. For the further details of the hypothesis one should consult Forbes' original article. Is it not conceivable that rhythmic alternation of inspiratory and expiratory activity might be explained in a somewhat similar way?

Apneusis consists essentially in a more or less prolonged failure to interrupt inspiratory activity. It has been observed by Kroneker and Markwald (1880), Lumsden (1923), and Keller (1931) as a result of pontile transection, by Henderson and Sweet (1929) as a result of section of the rubrospinal tracts plus section of the vagi, and by Taylor (1930, 1931) as a result of gradual poisoning by hydrocyanic acid gas. We have observed it as a result of severe cooling of the obex or slight cooling plus blocking of the vagi. All of these procedures might conceivably reduce

the frequency of impulses arriving at some internuncial neurone in the inspiratory pathway. It seems quite likely that the central respiratory mechanism receives impulses from various higher centers via the descending tracts of the brain stem as well as from many peripheral receptors via various afferent nerves such as the vagus and trigeminal. There is of course known to be an increase in impulse frequency in the vagi during

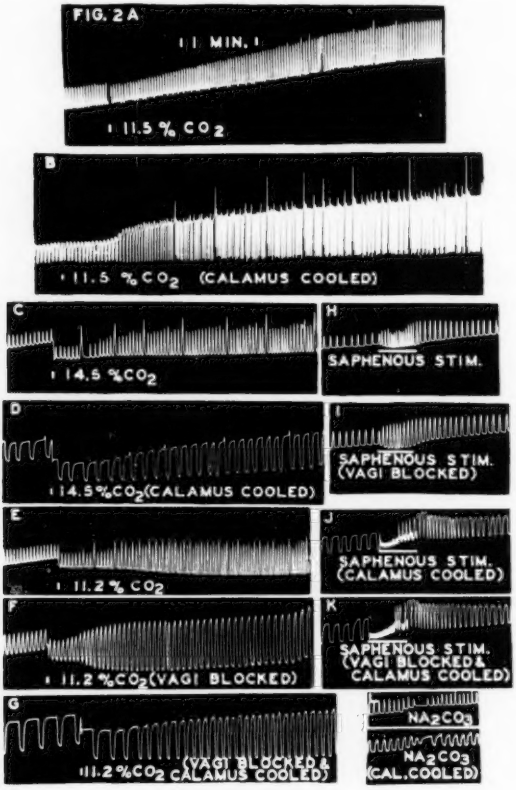

Fig. 2

normal inspiration. It does not seem necessary to assume, as has Lumsden, the existence of a special center in the upper pons with the specific function of periodically interrupting inspiration in order to explain apneusis as a result of pontile transection. If we simply assume that with the progress of inspiration the frequency of impulses arriving at some internuncial neurone in the inspiratory pathway normally reaches the critical frequency at which inhibition occurs but that following any of the

above mentioned procedures it is unable to do so we have an explanation for apneusis and also for normal interruption of inspiration.

Carbon dioxide administered intratracheally is of course an effective respiratory stimulant. Ordinarily, however, the augmentation of respiration produced by carbon dioxide consists principally of an increase in amplitude. Although the respiratory rate may increase somewhat it not infrequently remains unchanged or even decreases. With the vagi sectioned a decrease in respiratory rate is the usual effect of carbon dioxide administration. Administration of morphine also seems to render carbon dioxide particularly apt to produce slowing of respiration. However, if carbon dioxide is administered during local cooling of the calamus region there frequently results a tremendous acceleration of respiration. In figure 2 B is seen the effect of the administration of 11.5 per cent carbon dioxide in room air during such local cooling. In this case the respiratory rate was increased from 19 to 62 per minute, 226 per cent. This effect may be compared with figure 2 A showing a previous administration of the same carbon dioxide mixture with the brain stem at normal temperature. In figure 2 A the respiratory rate decreased about 3 per cent, from 28 to 27 per minute. The increase in respiratory amplitude was also considerably greater during cooling than at body temperature. However, cooling did not always have this effect of greatly increasing the effectiveness of carbon dioxide as an accelerator of respiration. In many instances the effect of carbon dioxide on respiratory rate was altered very little or not at all by cooling. When these experiments were examined it was found that there was a marked difference in the respiratory response to cooling in the two cases. In all of the experiments in which cooling greatly increased the effectiveness of carbon dioxide as a respiratory accelerator it was found that cooling alone markedly shortened or completely abolished the expiratory pause while prolonging the inspiratory phase of respiration only slightly or moderately. If as a result of cooling inspiration was greatly prolonged resulting in a marked slowing of respiration with well developed apneustic breathing then carbon dioxide was little if any more effective in increasing respiratory rate than in the absence of cooling. Figure 2 C and D shows the effects of administration of 14.5 per cent carbon dioxide in room air (C) with the brain stem at body temperature and (D) during local cooling of the calamus region. In this case as may be seen the cooling has caused well developed apneusis with marked slowing. In C the respiratory rate increased from 16 to 20½ per minute, about 28 per cent. In D the rate increased from 5 to 7 per minute, 40 per cent. Although the percentage increase in the latter case was somewhat greater the absolute rate was considerably less.

In figure 2 E and F is seen the effects of 11.2 per cent carbon dioxide administration with the vagi intact and with them blocked. In both

cases the calamus is at body temperature. In E with the vagi intact the respiratory rate increased from 24 to 32 per minute, 33 per cent. In F, with the vagi blocked the rate decreased from 19 to 12½ per minute, a 34 per cent decrease. Finally in figure 2 G is seen the effect of administration of the same carbon dioxide mixture with the vagi blocked and the calamus cooled. Under these conditions the respiratory rate increased from 4½ to 11½ per minute, about 155 per cent. It is noted though that the maximum rate during CO_2 administration is still somewhat less than in the case of figure 2 E.

These variations in the effectiveness of carbon dioxide as a respiratory stimulant are not easily explained. Apparently the integrity of the vagi are essential for a marked increase in respiratory rate from carbon dioxide administration. If figure 1 F be examined it is seen that with the vagi blocked carbon dioxide increased the amplitude of respiration considerably with no increase in the speed with which inspiration or expiration proceeded and a definite prolongation of the expiratory pause. Gesell and Moyer (1935) have demonstrated depression of various respiratory reflexes by carbon dioxide, an observation which we have verified as regards the respiratory responses to inflation and deflation of the lungs, and they suggest that the relatively slow breathing from carbon dioxide administration results from depression of the accelerating function of the vagi. Similarly the failure of carbon dioxide to cause marked acceleration of respiration during apneustic breathing produced by cooling is very likely due to depression of vagal function by the cooling. But how may we explain the extreme acceleration of respiration seen in figure 2 B? Of course here the cooling has doubtless not been sufficient to depress vagal function but why should the carbon dioxide be more effective than under normal conditions when of course there has also been no depression of vagal function? When one examines figure 2 B he sees that every phase of respiration has been accelerated. The active phases of both inspiration and expiration have been greatly speeded and every sign of a pause at the end of either phase has disappeared. About all we can say is that some factor which normally opposes the tendency of carbon dioxide to accelerate respiration has been eliminated. Whether this consists in the depression of some "center" with an inhibitory function, the elimination of some reflex or group of reflexes or what the exact mechanism involved is, we do not know. We have shown that moderate cooling may abolish the retarding effect of inflation of the lungs upon respiration while leaving unaffected or even enhancing the accelerating effect of deflation. It is possible that some such process as this may be involved, affecting vagal or other stretch reflexes.

Intravenous injection of sodium carbonate ordinarily decreases respiratory amplitude and may produce complete apnea. Gesell and Moyer

(1935) have shown that with smaller doses than those required to produce apnea, the effects on rate varied markedly with lung volume. Carbonate injection during inflation of the lungs slowed respiration, during deflation accelerated it, while at neutral volume there might be no effect on rate. They have concluded that carbonate acts to augment the prevailing reflex and that carbonate apnea is not due to lost excitability, but most probably to augmentation of vagal inhibitory reflexes and possibly other stretch reflexes. The effects of carbonate injections during cooling of the calamus region differed in two important respects from those observed in the absence of such cooling. First, with doses large enough to produce apnea the apnea was inspiratory rather than expiratory. Figure 2 L shows first respiratory arrest in expiration as a result of Na_2CO_3 injection with the calamus at body temperature and later a prolongation of inspiration resulting from injection of the same amount of carbonate with the calamus cooled. Second, with smaller doses carbonate was more apt to cause acceleration and less apt to cause slowing than normally. Since cooling favors inspiration over expiration the above results seem to support the view of Gesell and Moyer that carbonate augments the prevailing reflex, in this case the inspiratory.

Strong faradic stimulation of the central end of the sectioned saphenous nerve, or for that matter of almost any cutaneous nerve, ordinarily results in an acceleration of respiration. However, if the saphenous nerve is stimulated during cooling of the calamus region the acceleration is much more marked than occurs in the absence of such cooling. This increase in the effectiveness of saphenous stimulation is observed whether the cooling is moderate or extreme in degree with the production of apneusis. This is rather surprising in view of the fact that marked cooling eliminates vagal influence upon breathing and Gesell and Moyer (1935) have found that blocking the vagi *decreases* the effectiveness of saphenous stimulation. Figure 2 H, I, J and K shows the effects of faradic stimulation of the saphenous nerve with a stimulus of constant strength; (H) at body temperature with the vagi intact resulting in an acceleration of respiration from 12 to 60 per minute; (I) at body temperature with the vagi blocked with an increase in respiratory rate from 11 to 21 per minute; (J) during cooling of the calamus region with an increase in rate from 8 to 138 per minute, and (K) during cooling of the calamus with the vagi blocked with an increase in respiratory rate from 8 to 126 per minute. We believe, as we have already pointed out that the vagi are very important in normal respiration in terminating inspiration, and also in increasing the speed with which inspiration proceeds. As regards the effect of cooling upon saphenous stimulation it appears that the cooling has again, as in the case of carbon dioxide administration eliminated some factor which ordinarily opposes the tendency toward acceleration. However, the effect

of cooling upon saphenous stimulation differs in one important respect from its effect upon carbon dioxide administration. Whereas the marked accelerating effect of carbon dioxide during cooling was abolished by vagal block or extreme cooling (the latter apparently involving vagal depression) the acceleration of respiration from saphenous stimulation was most marked during apneustic respiration and was not appreciably diminished by vagal block. Furthermore, if one examines figure 2 J and K he sees that a very important factor in the acceleration of respiration is the very quick interruption of inspiration. That is, the increased rate and decreased amplitude appear to go hand in hand.

The respiratory stimulation produced by intratracheal administration of low oxygen mixtures was not importantly modified by cooling. The same was true of the stimulation resulting from intravenous injection of sodium cyanide except that in some cases the stimulation was somewhat more prolonged during cooling. In view of the fact that carbon dioxide is believed to exert its stimulating effect mainly centrally while low oxygen and cyanide act largely peripherally upon the carotid gland it is not surprising to find that the actions of the latter two are not affected by central cooling in the same way as is the action of carbon dioxide. Saphenous stimulation is of course an example of a peripheral stimulus whose effect is enhanced by central cooling. The stimulus, however, differs considerably in type from that provided by low oxygen and cyanide.

SUMMARY

The relationship of the vagus nerves to respiration and the modification of this relationship by local cooling of the floor of the fourth ventricle in the region of the calamus scriptorius was studied in various ways. The effects of faradic stimulation of the central end of the vagus upon respiration were markedly diminished by such cooling. During severe cooling of this region both inflation and deflation of the lungs were entirely without effect upon respiration. If the cooling was only moderate the retarding effect of inflation might be abolished while the accelerating effect of deflation was unaffected or enhanced.

During apneustic respiration resulting from severe cooling of the calamus region blocking the vagi was entirely without effect, indicating that this severe cooling had abolished all vagal effect upon breathing.

If the cooling was only slight in degree so that instead of apneusis there resulted merely a decrease in the expiratory pause with an acceleration of respiration, then blocking the vagi produced apneustic respiration. Apneustic respiration as seen in these experiments then is apparently the result of removal of vagal influence plus depression of some portion of the central respiratory mechanism. A sufficient amount of depression may be produced by lowering the temperature only a few degrees centigrade.

It is suggested that interruption of inspiration in normal breathing may be brought about by a process similar to that proposed by Forbes to explain reciprocal innervation and intrinsic alternation of flexion and extension, namely, by a rise in the frequency of impulses arriving at some internuncial neurone in the inspiratory pathway above a critical value. Apneusis then will result from any procedure which prevents or greatly delays this rise to the critical value. Since the central respiratory mechanism doubtless receives impulses from many different sources, peripheral receptors as well as higher nervous centers, it seems likely that apneusis might result from a variety of procedures, such as section of the pons (Kroneker and Markwald, 1880; Lumsden, 1923; Keller, 1931), section of the rubrospinal tracts plus section of the vagi (Henderson and Sweet, 1929), administration of HCN gas (Taylor, 1930, 1931), severe cooling of the floor of the fourth ventricle or slight cooling plus vagal section (the present authors). It does not seem necessary to assume the existence of a special center with the specific function of interrupting inspiration.

Intratracheal administration of a carbon dioxide mixture which with the brain stem at body temperature has no effect on respiratory rate, during *moderate* cooling of the calamus region caused a tremendous acceleration of respiration. If the cooling of the calamus region was severe so that apneustic respiration resulted this did not occur. Possible explanations of these findings are considered.

A dose of sodium carbonate which under normal conditions caused apnea in expiration during local cooling of the calamus region caused arrest of respiration in inspiration, apparently supporting the view of Gesell and Moyer (1935) that carbonate acts to augment the prevailing reflex.

Faradic stimulation of the saphenous nerve during local cooling of the calamus region whether this cooling is moderate or extreme in degree and whether or not the vagi are intact caused a much greater acceleration of respiration than in the absence of such cooling.

The respiratory effects of intratracheal administration of low oxygen mixtures and of intravenous administration of sodium cyanide were not significantly altered by local cooling of the floor of the fourth ventricle.

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EFFECTS OF ADMINISTERING ADRENOTROPIC EXTRACT TO HYPOPHYSECTOMIZED AND THYROIDECTOMIZED TADPOLES

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Several observers have reported that hypertrophy of the adrenal cortex follows administration of anterior pituitary extracts or solutions. The pertinent question has been raised as to whether this action upon the adrenal gland is exerted through the mediation of the thyroid.

Evans (7) noted that the adrenal cortex was sometimes hypertrophied in his hyperpituitary giant rats. Reiss and Langendorf (14) found enlargement of the adrenal in one of their pituitary treated dogs. Hypertrophy of the adrenal cortex following treatment with anterior pituitary extract has been observed by Emery and Atwell (5) in albino rats, by Anselmino, Hoffmann and Herold (2) in castrated female mice, and by Houssay and co-workers (8) in dogs. Loeser (10) noted hypertrophy of the adrenal after administration of thyreotropic extract, which fails in thyroidectomized animals. He holds that there is also a direct action of the pituitary upon the adrenals. Methods for standardizing adrenotropic extracts using mouse units have been proposed (1) (9).

Emery and Winter (6) employing anterior pituitary extracts, and McQueen-Williams (11) with pituitary implants, failed to obtain adrenal cortical hypertrophy in thyroid-parathyroidectomized rats. McQueen-Williams more recently (12) has shown marked differences in the ratios of thyreotropic and adrenotropic content of rat and beef pituitaries. Winter and Emery (15) have found that thyroidectomy does not affect the degree of compensatory hypertrophy which normally follows the removal of one adrenal.

Using the purified adrenotropic extract of Collip, Anderson and Thompson (4), the writer (3) produced marked hypertrophy and increase of osmiophilic content of the adrenal cortex in hypophysectomized amphibia. The present paper reports the results of experiments planned to ascertain whether administration of adrenotropic extract will cause hypertrophy of the adrenal cortex in the absence of the thyroid and hypophysis in frog tadpoles.

One group of tadpoles of Rana pipiens was hypophysectomized.

Another group was hypophysectomized and thyroidectomized. In some cases the two operations were attempted at the same time. It was found more convenient, however, to remove the hypophysis on one day and the thyroid one or two days later, the tadpoles having been kept in the meantime at a temperature not exceeding 15°C. At the time when the hypophysis is most accessible (tail-bud stage or slightly later) the thyroid is not yet distinctly enough evaginated to permit complete extirpation without removal of considerable entoderm.

From these two groups animals which had survived five and one-half to six months and which showed the external signs of hypophysectomy, i.e., silvery color and failure to make progress towards metamorphosis, were chosen for treatment with adrenotropic hormone. No further signs were observed by which absence of the thyroid, in addition to that of the hypophysis, could be recognized.

Adrenotropic extract supplied by Dr. J. B. Collip and labeled "Sept. 26, 1935, AI 33A," was administered according to the technique previously described (3). Using special fine steel needles 0.05 cc. of the extract was injected intraperitoneally, six days a week for 20 injections. The animals were autopsied on the following day. A few animals were killed following 13 or 19 injections.

For ease of description the animals are divided into three groups: A, 11 animals hypophysectomized and treated with adrenotropic extract; B, 17 hypophysectomized and thyroidectomized, treated with adrenotropic extract; and C, 27 hypophysectomized and thyroidectomized, no treatment. At autopsy, records of total length, body length, hind leg and front leg length were made. The following regions were examined under the binocular microscope and conditions noted: thyroid, hypophysis and adrenal-gonad. The first two were preserved in Bouin's fluid and the last in chromic-osmic. All were later cut into serial sections and appropriately stained. In addition to these three groups of animals there were available for study similar records and serial sections from numerous other tadpoles: normal, hypophysectomized, untreated and treated with adrenotropic or thyreotropic extract.

Study of the hypophysis region, both at autopsy and after the preparation of serial sections, showed complete absence of the epithelial hypophysis. The neural lobe was found in its usual location apparently somewhat smaller than normal.

Examinations of the thyroid region in the animals of group A where no attempt at thyroidectomy had been made, all revealed the thyroids present in their normal position. In the autopsy protocols the glands were noted as "medium-sized," "moderate in size" or "large." Study of serial sections showed various stages of repair or hypertrophy of the thyroids in contrast to the extreme atrophy found in untreated hypophysectomized

tadpoles (3). Apparently the adrenotropic extract employed possessed moderate power of stimulating the thyroid. Probably this was due to contamination with a small amount of thyreotropic hormone. That such stimulated thyroids were physiologically active is indicated by the increase of hind leg length and by other progress towards metamorphosis made during the period of treatment.

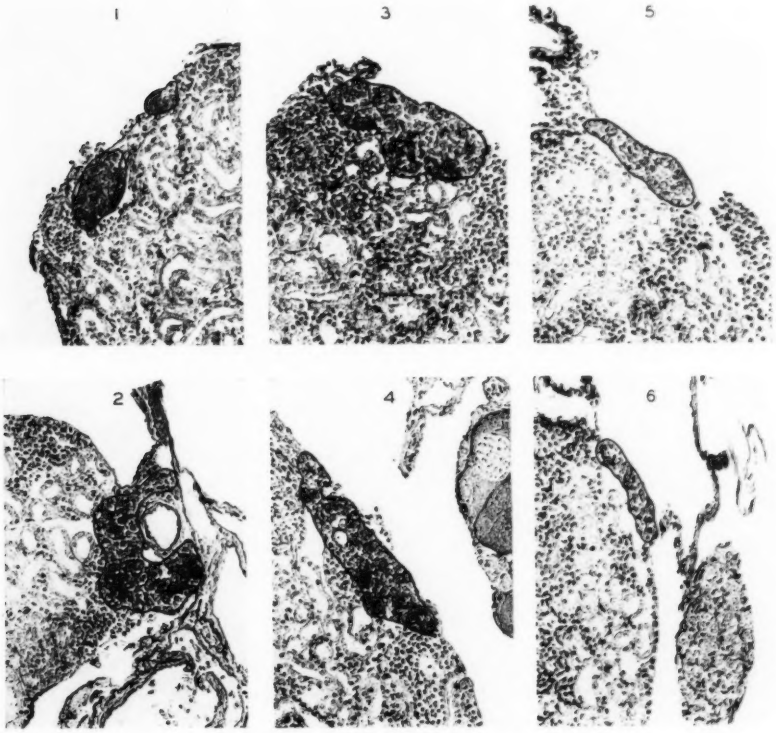
In the animals of groups B and C, where complete thyroidectomy, in addition to hypophysectomy, had been attempted, autopsy occasionally showed fragments of the thyroid. Sometimes these were found in the normal position, sometimes more rostral. Careful searching in serial sections extending from the base of the tongue to the heart revealed, in a few cases, small remnants not visible at autopsy. Classification of an animal as completely thyroidectomized was made only after thorough study had shown entire absence of thyroid tissue. Four such animals were found in group B and seven in group C.

Animals in which remnants of the thyroid were found provided valuable controls. In group C microscopic study of these remnants showed them all to be atrophic with very flat epithelium. No matter what the size of the remnant, whether as large as one entire thyroid under such conditions, or only a minute fragment, no compensatory hypertrophy could be discovered. Apparently compensatory hypertrophy of thyroid remnants does not occur in hypophysectomized tadpoles. This is in accord with the recent observations of Magdalena (13) on the adult toad.

The thyroid remnants found in certain animals of group B (hypophysectomized, thyroidectomy attempted, adrenotropic treated) all exhibited some stimulation as shown by tall epithelium and other evidences of hypertrophy. The physiological activity of these stimulated thyroid remnants was shown by increase of hind leg length from an average of 3.5 mm. at the beginning to 8.1 mm. at the end of treatment. The hind legs of completely thyroidectomized tadpoles averaged 3.9 mm. at the close of the treatment period.

The adrenal glands were studied during the process of clearing in chloroform, where the osmiophilic material contained in the cortical cells could be seen clearly, and later in serial sections counterstained with Babe's safranin. The adrenals of the animals of group C were all very small and poor in osmiophilic content (figs. 5 and 6). They resemble the adrenals of untreated hypophysectomized tadpoles (3). Complete thyroidectomy, in addition to hypophysectomy, does not increase the degree of adrenal atrophy over that found after hypophysectomy alone.

In group B (hypophysectomized, thyroidectomized and adrenotropic treated) the adrenals were large and contained much osmiophilic material (figs. 3 and 4). This reaction is fully as great as in animals which were only hypophysectomized prior to treatment (figs. 1 and 2). The absence



All figures are photomicrographs made from largest transverse section of one adrenal gland and adjacent structures in larvae of Rana pipiens and here reproduced at × 85. The adrenal gland has been outlined in each instance. Tissues were fixed in chromic-osmic and serial sections were stained with Babe's safranin.

Figs. 1 and 2 are from animals hypophysectomized for 181 days and then treated with 13 and 19 injections, respectively, of adrenotropic hormone (Collip).

Figs. 3 and 4 are from animals hypophysectomized and thyroidectomized for 158 days and then treated with 20 injections of adrenotropic hormone.

Figs. 5 and 6 are from animals hypophysectomized and thyroidectomized for 186 and 184 days, respectively. No treatment.

Figs. 5 and 6 show the atrophic condition of the adrenal which follows hypophysectomy.

Figs. 1 to 4 show the hypertrophy and increased content of osmiophilic material which follow administration of adrenotropic hormone. These changes are fully as marked in the animals which were both hypophysectomized and thyroidectomized (figs. 3 and 4) as in those hypophysectomized only (figs. 1 and 2).

of the thyroid has not prevented the repair, even hypertrophy, of the atrophied adrenal glands. Conversely, the possession of thyroid fragments by some of the animals has not increased the degree of adrenal response to treatment.

CONCLUSIONS

Administration of adrenotropic extract to tadpoles of Rana pipiens which had been both hypophysectomized and thyroidectomized resulted in hypertrophy of the atrophic adrenal glands and great increase in their content of osmiophilic material (figs. 3 and 4).

This reaction is fully as great as that obtained when the thyroid gland is entirely present (figs. 1 and 2), or when thyroid fragments remain after incomplete removal.

In hypophysectomized tadpoles there is no compensatory hypertrophy of the thyroid fragments remaining after unsuccessful thyroidectomy.

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THE RATE OF LACTIC ACID REMOVAL IN EXERCISE

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The rate of removal of lactic acid from the body of a man resting after exercise is directly proportional to the amount present, according to the work of Margaria, Edwards and Dill (12, 13, 14, 5). The following equation is given by them:

$$\log L = a - bt \tag{1}$$

relating milligram per cent of lactic acid in arm vein blood, L, to recovery time t in minutes. The velocity constant, b, is the slope of the straight line relating log L to time, t (fig. 1B). That the rate of removal of lactic acid from the whole body is the same as from the blood was shown by Margaria and Edwards by analyzing the whole bodies of mice. The same value of b, 0.02, was found for resting man and for mice (13). In other words, one-half of the lactic acid present at the end of exercise will be removed in 15 minutes.

The above equation is of the type which represents an apparent monomolecular reaction. If one were dealing with a simple physicochemical system, the factors responsible for the velocity constant could be measured. A general theory of reaction velocity in heterogeneous systems by Nernst (18), based upon the assumption of slow diffusion of products and reactants but a rapid reaction at the interfacial layer, presents the factors in a velocity constant for that type of reaction.

According to Nernst, the velocity constant b is directly proportional to a diffusion coefficient representing the amount of reactant translated to the reacting surface in unit time per unit concentration. The analogy of the removal of lactic acid in the body to a simple physico-chemical reaction system is obviously loose but furnishes the hint that the rate of removal of lactic acid from the body of a resting man may be limited by the slow rate of translation of products and reactants in the resting body. An attempt is made in our present experiments to vary this factor in the body by varying the circulatory and vascular commotion.

From a physiological standpoint lactic acid has a restricted rôle in muscular exercise in the opinion of Margaria, Edwards and Dill (12). In principle Bang concurs in this opinion (1). Moderate exercise may

not involve the production and removal of lactic acid; it may end with the concentration of lactic acid at the rest level. The initiation of such work brings into play an alactacid process which can account for about 3 litres of oxygen debt. Lactic acid is produced by the muscles when the work is severe enough to require a greater anerobic liberation of energy than the alactacid process can furnish. In recovery the accumulated lactate, constituting part of the oxygen debt of the work, is removed. The question arose, what would happen to the rate of removal of lactic acid previously accumulated as a debt when work was subsequently performed at levels of oxygen consumption which do not raise the lactic acid level?

EXPERIMENTAL. Each of the three subjects of these experiments came

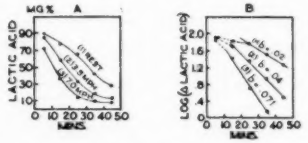

Fig. 1A. The removal of lactic acid in rest compared with its removal while walking at 3.5 miles per hour and running at 7 miles per hour. At time zero the subject had completed about 1 minute's anaerobic work. Moderate work was begun in two cases after 5 minutes' rest.

Fig. 1B. The results of figure 1A arranged to show the relation of the log of lactic acid increment to time. The slope of this line, *b*, measures the velocity of lactic acid removal.

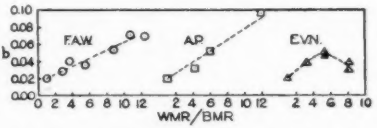

Fig. 2. The velocity of lactic acid removal in relation to WMR/BMR, i.e., to the ratio of work metabolism to rest metabolism. Subjects F.A.W. and A.P. are athletic, E.V.N , non-athletic.

to the laboratory in the morning without breakfast and rested on a bed for 20 minutes. He then ran on a motor-driven treadmill at 18.7 km. per hour on a 12 per cent grade until nearly exhausted (45 to 65 seconds). After the subject rested 5 minutes on a bed, a sample of venous blood was drawn and work of a more moderate nature was undertaken on the treadmill. This was continued for 45 minutes and during this time 4 or 5 more samples were drawn from the arm vein, the work being interrupted about 30 or 40 seconds for the drawing of each blood sample. The blood was analyzed for lactic acid concentration by the method of Friedemann, Cotonio and Schaffer (8) with some unpublished modifications introduced by Edwards of this laboratory.

RESULTS. After lactic acid becomes uniformly distributed throughout body fluid, its concentration in the blood is proportional to the total amount in the body (12, 13). Hence its rate of removal from the body can be followed by determining the concentration of blood lactic acid at various times. Figure 1 contains results of three typical experiments. In part A, blood lactic acid concentration is plotted against time elapsed since completing the preliminary short exhausting run; in part B, the logarithm of the blood lactic acid (in excess of the resting level, 10 mgm. per cent) has been plotted against time. The slope of this line is b, the velocity constant in equation (1). The first point on these curves does not fall on the logarithmic line. Being taken before moderate work begins, it is not comparable to points later when the metabolic state of the body is at a steady higher pitch. Five to 10 minutes are necessary for the processes involved in determining the logarithmic curve to equilibrate and reach this definite course.

By treating the data for each experiment as in figure 1, the velocity constant b has been determined. In figure 2, b has been plotted against the metabolic rate expressed as the ratio

$$\frac{\text{Work metabolic rate}}{\text{Basal metabolic rate}} = \frac{\text{WMR}}{\text{BMR}}$$

It is evident that the velocity constant is approximately proportional to the metabolic rate up to some critical level of activity. For subject F. A. W., a trained runner, the constant was increased 3.5-fold the value at rest. For the subject A. P., a former trackman, capable of exceptional performances in former laboratory runs, it rose nearly 5-fold. Both these men were able to carry out these rates of work for 45 minutes with no increase in blood lactic acid, since at the end of a run its concentration was at the rest level. The subject E. V. N., non-athletic, could not carry out work for 45 minutes at an oxygen consumption much greater than 5 times basal without an increase in blood lactate. The velocity constant for this man increased to 2.5-fold when $\frac{\text{WMR}}{\text{BMR}}$ was 5.3 and then subsequently declined.

To convert the velocity constant into terms of physiological meaning requires short consideration of the relation given in equation (1). In different form, the rate of removal of lactic acid is proportional to the amount present at any time, as follows:

$$\frac{dL}{dt} = -bL \tag{2}$$

However, if one is comparing velocity constants b under different physiological conditions, at a given concentration of lactic acid L, it is plain that

the rate of removal of lactic acid $\frac{dL}{dt}$ is directly proportional to the velocity constant. Thus, the relations in figure 2 show that the ability of the body to remove excess lactic acid increases proportionately with the rate of work carried on.

INTERPRETATION. In seeking explanations for a more rapid removal of lactic acid in exercise, various factors suggest themselves as possibly significant. Loss through the channels of sweat and urine may be roughly estimated. Using the assumptions of Margaria, Edwards and Dill (12), that lactic acid is uniformly distributed throughout body water, with 80 per cent water in blood and 72 per cent in the whole body, the total amount of excess lactic acid present in the body of the subjects after the standard exhausting run was about 50 to 60 grams. Making the generous estimate that in 45 minutes of work a litre of sweat is produced, with 0.1 per cent of lactic acid, one gram may be excreted through this channel (19, 21). The quantity found in urine after exercise rarely exceeds 1.0 gram (7, 20). Thus the quantity of lactic acid which can be excreted (about 2 grams) is an unimportant fraction of the total to be removed (50 to 60 grams).

The heart uses lactic acid for fuel (6, 16, 17); according to McGinty and Miller (17), nearly one-half of the oxygen used by the heart may be for the oxidation of lactic acid. Various estimates of the oxygen consumption of the heart have been made, ranging from 10 cc. per minute in rest to 160 cc. per minute in maximal work (6, 10, 16). Assuming that the heart used for the oxidation of lactic acid 100 cc. of oxygen per minute for 45 minutes of the highest grade of aerobic work of the present experiments, not more than 5 grams of lactic acid would be oxidized. This is only 10 per cent of the estimated total to be removed from the body.

Having roughly evaluated the excretory removal of lactic acid and its use by the heart, one must turn to other changes occurring in exercise which may be responsible for the increased rate of removal of lactic acid. The limiting factor may be the rate of translation of lactic acid to reactive centers. This in turn depends on the circulatory rate, a function which is known to have a linear relation to oxygen consumption in exercise (2, 3, 4, 11, 15).

Finally it is not improbable that one reactive center is the skeletal musculature used in the period of aerobic work. An economy might thus be effected in paying the oxygen debt, since the conversion of lactic acid to glycogen is moderately expensive. This expense is part of the oxygen debt of anerobic work if payment is made during complete rest. On the other hand, if the debt is paid during moderate work, the expense of resynthesis may be reduced or eliminated. Preliminary experiments support this hypothesis but at present technical difficulties have prevented satisfactory quantitative measurements.

The conception of the steady state (2, 9, 10) has altered greatly in recent years. A notable contribution in this field has been made recently by Bang (1). According to his hypothesis, no lactic acid formation occurs in exercise except in the first minutes. For moderate work in which the increase, if it occurs, is transitory, his hypothesis is tenable. For severe work which is carried on with sustained high values of lactic acid, whether constant or not, his hypothesis is open to question. In such activity, muscles carrying a small share of the burden may carry on aerobically while others may have to work in part anaerobically. If the proportion of the latter is small, a steady state may be attained; if large, lactic acid may increase to the point of exhaustion. We see no proof in Bang's experiments that lactic acid is not being produced continuously under such circumstances. On the other hand, our finding that the rate of removal is greater in moderate exercise than in rest supports the older idea of a balance between formation and removal.

SUMMARY

Three subjects, having run previously to exhaustion in 1 minute, worked on a treadmill at rates up to 12 times the basal rate of oxygen consumption for 45 minutes. With one exception the 45 minutes of work did not itself cause increased blood lactic acid.

Previous reports that rate of removal of lactic acid from the body in exercise is a logarithmic function of time have been confirmed.

The rate of removal of lactic acid in exercise increases approximately proportionately with the metabolic rate up to some critical level of activity, different for each subject.

The increased rate of removal in exercise is interpreted as being due to two possible factors: 1, an increase in blood flow proportional to metabolic rate with more rapid transfer of lactic acid to reactive centers, and 2, the use of lactic acid as a fuel for the work.

We are indebted to Arthur Pier for his generous coöperation as subject in these experiments.

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ON THE PREPARATION AND BIOLOGICAL ASSAY OF ENTEROGASTRONE

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The existence of a chalone (enterogastrone) mechanism for the inhibition of gastric secretion and motility following the ingestion of adequate quantities of fat or sugars has been demonstrated by several groups of investigators (1–11). The purpose of the present investigation was to evolve a more efficient and dependable method for the preparation of enterogastrone in large quantities, to develop an accurate method for its assay, to investigate its properties, and finally, to investigate the cause of the variations in response in the same and different animals noted in preliminary studies.

METHODS OF ASSAY. In assaying various preparations of enterogastrone, its effects on gastric motility and secretion were studied. Its effect on two types of gastric contraction was determined, namely, hunger contractions and contractions initiated by distention of the empty, fasting stomach with a balloon. Only adequately trained dogs conditioned to all types of hypodermic injections were employed. To insure good gastric tone and prolonged periods of hunger motility the animals were fasted 48 hours. To initiate contractions by distention, the recording balloon in the stomach was distended with from 80 to 100 cc. of air. In order to investigate the action of preparations of enterogastrone on gastric secretion, dogs with a Pavlov pouch, or a pouch of the entire stomach (12) were used.

METHODS OF PREPARATION OF ENTEROGASTRONE. *Picric acid methods.* Lim's (8) later picric acid method was tried up to the stage of sodium carbonate precipitation. A variation of this, Dodds and Dickens' (13) picric acid method for insulin production, was also tried. It was found that neither of these methods was satisfactory for preparing large quantities of extract and they possessed the further disadvantage of being relatively inefficient (table 1).

The sodium chloride precipitate or "A precipitate" method. This method (14) has been used for the preparation of crude secretin. It has proved

to be very satisfactory for obtaining a crude preparation of enterogastrone, practically free of vaso-depressor substances.

Tannic acid method for concentrating enterogastrone. Many different methods of purifying the crude "A precipitate" were tested.

The most successful method for obtaining potent preparations regularly was based on the precipitation of an aqueous extract of "A precipitate" with tannic acid. Four hundred grams of "A precipitate" were suspended in one liter of water and sufficient normal sodium hydroxide added to bring the reaction to the neighborhood of pH 5.5. The solution was boiled for one minute and filtered while still hot. The coagulum was resuspended in one liter of fresh water, boiled, and filtered. To the combined filtrates, which had been cooled under running water, 50 grams of tannic acid (resin-free) were added; and the resulting flocculent precipitate was collected in large centrifuge cups. The precipitate was washed with distilled water and recentrifuged until packed hard in the centrifuge cup. Absolute acetone was added in a volume somewhat greater than that of the precipitate, so that the final concentration was about 60 per cent. The precipitate was broken up and 15 drops of concentrated hydrochloric acid added to split off the tannic acid. The insoluble, inactive portion was removed by rapid centrifugalization. This insoluble portion was reëxtracted with a small quantity of 60 per cent acetone, and the supernatant fluids combined. The active substance was precipitated by adding 5 to 7 volumes of acetone, washed once with acetone, twice with methyl alcohol, and once again with acetone. After evaporating most of the acetone, the contents were spread on paper in the air until all traces of organic solvents had disappeared. The yield is about 2.8 grams from 400 grams of "A precipitate."

TABLE 1

Comparative yields by different methods of preparation of enterogastrone

PREPARATION	YIELD FROM 100 GRAMS MUCOSA	DOSE—GIVING 6 MINUTES INHIBITION	NUMBER OF DOSES PER 100 GRAMS MUCOSA
Lim's picric acid method	750 mgm.	50 mgm.	15
Dodd and Dickens' picric acid method	200 mgm.	30 mgm.	7
Secretin "A precipitate" method	14.0 grams	300 mgm.	47
Tannic acid method	100 mgm.	3 mgm.	33

This product has been fractionated by ethyl alcohol, isoelectric precipitation at pH 8.4, pyridine precipitation, phenol extraction, and picric acid precipitation, but so far none of these methods has proved satisfactory.

RESULTS. *Gastric motility.* The effect of enterogastrone preparations

on the two types of gastric motility studied is practically the same. A well-defined inhibition of motility lasting for approximately 6 minutes is consistently obtained with 0.2 mgm. per kilo of different batches of the tannic acid preparations. In table 2 are shown data (averages on three dogs) pertaining to the relation between the duration of inhibition of gastric motility and the dose of enterogastrone, the latter being expressed in gastric secretory units (vide infra).

Although the effect of our preparation is practically the same on the two types of motility, one obtains more consistent results on the motility excited by distention of the stomach with a balloon. This is apparently due to the fact that, in contrast to hunger contractions, the motility initiated by distention is more regular and rapid, shows a more constant tonus level, and is not subject to spontaneous rest periods. As a result, a number of assays may be made in several hours, with the certainty that a prolonged inhibition is not merely a spontaneous rest period.

TABLE 2

Showing the relation of dosage to the duration of the inhibition of gastric motility

	SECRETORY UNITS OF ENTEROGASTRONE												
	0.025	0.04	0.05	0.07	0.08	0.10	0.13	0.20	0.30	0.40	0.66	1.00	1.33
Inhibition in minutes.....	4.2	5.7	6.4	6.2	7.7	8.3	8.3	13.1	19.0	25.0	29.8	52.4	64.3
Number of assays.....	3	5	7	2	1	5	2	4	3	3	2	3	1

This assurance that the stomach will not undergo spontaneous changes in motility, made it possible to determine *the effect of subcutaneous injections* of enterogastrone preparations. It was found that four gastric secretory units given subcutaneously inhibited movements of the stomach for approximately one hour. Smaller doses had very little effect.

The possibility of obtaining non-specific inhibition of motility was checked in a number of ways, namely, by injecting inactive preparations containing similar quantities of foreign matter, by injecting salt solution, by injecting inactive material containing a small amount of histamine (0.1 mgm.), and by injecting toxic fractions. The histamine containing solutions caused only a momentary inhibition. The toxic fractions caused marked respiratory changes and completely abolished gastric motility for a prolonged period. The enterogastrone preparations show neither of these reactions. The innumerable injections of inactive, non-toxic fractions served as more than a necessary number of controls.

Gastric secretory assays using dogs with pouch of the entire stomach. Earlier in our work we used Pavlov pouch dogs which had been fed a meal or injected with histamine. Although when using an active preparation in sufficient amounts, an inhibition could always be obtained, a quan-

titatively uniform response could not be obtained. This, at first, led us to suspect that either some sort of non-specific reaction was concerned, or a variation in the secretory stimulus existed, in spite of the fact that only the best of pouch dogs were used. We then resorted to the use of dogs with a pouch of the entire stomach in which preparation the gastric vagi are sectioned and in which all the gastric mucosa is present to serve as a larger reacting surface. In using these animals we first established that the frequent injection of histamine was capable of producing a constant rate of secretion of gastric juice, even though continued for many hours. In observing this absence of fatigue in the gastric glands, we confirm Ivy (15) and Lim (16). It was soon found that the effect of a uniform dose of enterogastrone depends on the rate of flow of gastric juice prior to the injection. The examples of tests given in table 3 serve as examples

TABLE 3

Relation of histamine and rate of secretion to enterogastrone inhibition produced by one unit of enterogastrone

DOG	HISTAMINE PER 10 MINUTES	CONTROL		2 HOURS POST ENTEROGASTRONE			
		Per minute	HCl	Volume	Inhibition	HCl	Inhibition
	mgm.	*cc.*	*mgm. per minute*	*cc. per minute*	*per cent*	*mgm. per minute*	*per cent*
F	0.1	1.91	9.73	1.72	8	8.79	11
F	0.1	1.61	8.65	1.24	23	6.22	28
F	0.05	1.39	7.07	0.85	39	4.19	41
F	0.1	1.21	6.25	0.77	36	3.45	45
F	0.05	1.09	5.49	0.66	35	2.93	47
F	0.06	1.04	4.46	0.53	50	2.23	50
F	0.045	0.87	3.65	0.41	53	1.58	57
M	0.10	0.99	5.19	0.50	49	2.06	60
M	0.15	0.97	4.95	0.55	44	2.14	58

of this fact. Such results directly implied that the degree of inhibition varied oppositely to the control rate of secretion, and within limits, bears no relation to the quantity of histamine injected. Accordingly, in order to obtain consistent results, it was necessary to establish the same rate of secretion in all dogs and in the same dogs from day to day, by varying the dose of histamine. When this was done, the responses to the same preparation of enterogastrone became uniform. In order to maintain the secretion at a constant rate in the same and different dogs, a small dose of histamine (0.04 to 0.15 mgm.), which was adequate to cause the formation of approximately 1 cc. (5 mgm. HCl) per minute, was injected subcutaneously every 10 minutes. After such a dose of histamine has been determined for a dog, although still subject to some variation, the secretory level after one hour can be maintained with a ± 3 per cent variation per

hour, the variation from day to day being about ±10 per cent. After such a level has been maintained for one hour, the secretion is collected at 20 minute intervals for an additional "control" hour before the preparation to be tested is administered.

TABLE 4

Enterogastrone assay on total pouch dog receiving continuous histamine

TIME	VOLUME	MGM. HCl	MGM. HCl PER CC.	PARIETAL SECRETION	NON-PARIETAL SECRETION	MUCIN AS MGM. GLUCOSE
11:40	20.7	99.5	4.81	16.6	4.1	0.9
12:00	20.8	107.0	5.13	17.8	3.0	1.2
12:20	20.5	111.0	5.36	18.5	2.0	1.0
Hourly average...	20.7	105.8	5.10	17.6	3.0	1.0
1 unit of enterogastrone						
12:40	9.2	34.7	3.80	5.8	3.4	0.7
1:00	9.3	38.4	4.13	6.4	2.9	1.7
1:20	12.3	43.7	3.55	7.3	5.0	0.6
Hourly average...	10.3	38.9	3.77	6.5	3.7	1.0
1:40	9.5	31.9	3.36	5.3	4.2	0.3
2:00	9.7	50.2	5.18	8.4	1.3	0.6
2:20	10.0	48.3	4.83	8.0	2.0	1.0
Hourly average...	9.7	43.5	4.48	7.2	2.5	0.6
2:40	7.7	31.9	4.14	5.3	2.4	0.8
3:00	9.2	36.5	3.97	6.1	3.1	1.0
3:20	11.0	45.5	4.13	7.6	3.4	0.9
Hourly average...	9.3	38.0	4.09	6.3	3.0	0.9
3:40	11.3	48.3	4.27	6.1	5.2	
4:00	14.3	66.5	4.65	11.1	3.2	
4:20	21.4	93.0	4.35	15.5	5.9	
Hourly average...	15.7	69.3	4.41	10.9	4.8	
4:40	21.3	104.0	4.88	17.3	4.0	

In table 4 are recorded the results of a typical assay of enterogastrone using the above method. The enterogastrone during the two hours following its injection, reduced the volume of secretion by 49 per cent, and the acid output by 60 per cent. The inhibition, however, continued to be manifest for four hours.

Such a response constitutes slightly more than what we have arbitrarily designated as a tentative gastric secretory unit of enterogastrone. It

may be defined as follows: *A unit of enterogastrone* is that quantity, which when injected intravenously in a 12 to 14 kgm. dog with a pouch of the entire stomach receiving sufficient histamine subcutaneously at 10 minute intervals to maintain a uniform flow of 1 cc. of gastric juice (5 mgm. HCl) per minute, causes a 50 per cent reduction in the secretion of hydrochloric acid during two hours following its injection.

In this method of assay individual variations in response to the secretory stimulus have been completely compensated, and since it is apparently the secretory rate which determines the response to enterogastrone, the method might be expected to yield identical results in different animals. However, in the absence of sufficient proof that uniform results can be obtained in all animals, this unit must remain for the present a tentative one.

It was found that (table 4) the concentration of hydrochloric acid in the gastric juice was reduced to a greater extent than the volume of secretion. This might be explained on the basis that enterogastrone inhibits the acid-secreting cells without affecting other elements of the secretory apparatus. In order to investigate this possibility, the volume of secretion originating in the parietal cells was calculated and "non-parietal" secretion obtained by difference. According to Liu, Yuan and Lim (17) and Hollander (18) the oxyntic cells produce a fluid containing approximately 6 mgm. of hydrochloric acid per cubic centimeter. Accordingly, by dividing the total milligrams of hydrochloric acid by 6, one should obtain a figure which would represent the volume of "parietal secretion" necessary to contribute the total amount of hydrochloric acid. The remainder of the volume would be "non-parietal secretion" and would presumably represent mucous and pyloric secretion, or the hypothetical diluting secretion. This method is not intended to yield an accurate figure, since it does not take into consideration the basic character of the mucous and pyloric secretion, but the values obtained should be an approximate index of the "true" figures. In table 4 the volume of "non-parietal" secretion is seen to vary only slightly after the administration of enterogastrone. Further evidence to support this interpretation is afforded by the fact that the mucin content of the gastric samples parallels the very slight changes in "non-parietal secretion." The mucin values were obtained by the method of Anderson, Fogelson, and Farmer (19), which consists of estimating the reducing substances after acid hydrolysis of the mucin.

Upon administering four units of enterogastrone *subcutaneously* to total pouch dogs receiving continuous injections of histamine, no inhibitory effect has been obtained; on the contrary a slight stimulation of secretion has resulted. This is probably due to small amounts of histamine contained in these preparations.

For manifest reasons it was thought desirable to compare the inhibition caused by a unit of enterogastrone with the inhibition caused by olive oil administered orally. Typical results obtained after the administra-

TABLE 5

Effect of olive oil on secretion of total pouch dog receiving continuous histamine

TIME	VOLUME	MGM. HCl	MGM. HCl PER CC.	PARIETAL SECRETION	NON-PARIETAL SECRETION
11:40	22.0	107.0	4.86	17.8	4.2
12:00	23.0	116.0	4.95	19.3	3.7
12:20	24.1	119.0	4.94	19.8	4.3
Hourly average....	23.0	114.0	4.96	19.0	4.1
50 cc. olive oil into duodenum by tube					
12:40	21.0	104.0	4.96	17.3	3.7
1:00	17.6	88.0	5.00	14.7	2.9
1:20	17.8	87.3	4.91	14.5	3.3
Hourly average....	18.8	93.1	4.95	15.5	3.3
1:40	21.4	105.0	4.92	17.5	3.9
2:00	19.0	92.0	4.83	15.3	3.7
2:20	11.3	50.4	4.45	8.4	2.9
Hourly average....	17.2	82.5	4.80	13.7	3.5
2:40	16.5	76.0	4.61	12.7	3.8
3:00	16.5	77.4	4.69	12.9	3.6
3:20	17.9	77.8	4.34	13.0	4.9
Hourly average....	17.0	77.1	4.54	12.9	4.1
3:40	19.8	88.2	4.46	14.7	5.1
4:00	19.5	89.5	4.58	14.9	4.6
4:20	17.0	79.2	4.66	13.2	3.8
Hourly average....	18.8	85.6	4.55	14.3	4.5
4:40	18.5	85.6	4.62	14.2	4.0
5:00	20.9	95.6	4.57	15.9	5.0
5:20	23.2	112.4	4.85	18.7	4.5
Hourly average....	20.9	97.8	4.70	16.3	4.5

tion of 50 cc. of olive oil by tube to a total pouch dog are shown in table 5. Here the inhibitory action is not so great, there being only 22 per cent inhibition of volume and 26 per cent of hydrochloric acid. This is probably due to the rather rapid passage of the oil through the upper intestine

in these animals since, on several occasions, the oil leaked appreciably per anum about one hour after being given.

In table 6 are shown the results of four assays of the effect of orally administered olive oil on the gastric secretion of total pouch dogs. The average is approximately 28 per cent inhibition of hydrochloric acid. In the same table it will be noted that 0.5 unit of enterogastrone gives approximately 24 per cent inhibition, 1.0 unit about 50 per cent, 1.5 units 70 per cent, and 2.0 units 90 per cent inhibition of hydrochloric acid production.

TABLE 6

Inhibitory effect of olive oil and enterogastrone in total pouch dogs receiving continuous histamine

SUBSTANCE ADMINISTERED	DOG	CONTROL		TWO HOURS AFTER			
		Per minute	HCl	Per minute	Inhibition	HCl	Inhibition
		cc.	*mgm. per minute*	*cc.*	*per cent*	*mgm. per minute*	*per cent*
50 cc. olive oil	F	1.23	6.20	0.88	29	4.30	31
50 cc. olive oil	F	1.05	4.65	0.69	35	2.90	38
50 cc. olive oil	M	0.91	4.74	0.82	11	3.83	20
50 cc. olive oil	M	1.16	5.70	0.90	29	4.39	23
0.5 unit enterogastrone	F	1.06	5.10	0.87	19	4.01	22
0.5 unit enterogastrone	F	0.93	3.82	0.73	22	2.78	26
0.5 unit enterogastrone	M	1.15	5.69	1.04	10	5.01	12
1.0 unit enterogastrone	F	1.09	5.49	0.66	35	2.93	47
1.0 unit enterogastrone	F	1.04	4.46	0.53	50	2.23	50
1.0 unit enterogastrone	M	0.99	5.19	0.50	49	2.06	60
1.0 unit enterogastrone	M	0.97	4.95	0.55	44	2.14	58
1.5 unit enterogastrone	F	0.93	4.33	0.33	64	1.15	74
1.5 unit enterogastrone	F	1.44	7.24	0.57	62	2.52	65
2.0 unit enterogastrone	F	1.61	8.65	0.77	52	3.45	60
2.0 unit enterogastrone	F	1.14	4.90	0.21	83	0.46	91
2.0 unit enterogastrone	M	1.47	7.43	0.26	82	1.18	83

The assay of enterogastrone using Pavlov pouch dogs. The effect of enterogastrone on the secretion of Pavlov pouch dogs in response to a meal and to histamine was studied. The test meal consisted of 200 grams of ground beef heart freed from all visible fat and brought to a boil in 200 cc. of water. The enterogastrone when injected was given immediately after the meal. A control meal without was alternated with a meal with enterogastrone to provide a basis for comparing averages. When histamine was employed as a stimulus, it was administered either in a single dose (0.1 mgm. per kilo of histamine acid phosphate), or in the same way as used in the dogs with a pouch of the entire stomach (vide supra). When

the latter method was employed, the control level of secretion was maintained at a rate less than 1 cc. per minute. The portion of the total secretion which was formed by the pouch was estimated according to the size of the pouch in relation to the size of the main stomach, and the rate of secretion adjusted so that the total gastric secretion would be about 1 cc. per minute. This is obviously not as accurate as when the method is applied to the pouch of the entire stomach, because the relative sizes of the pouch and main stomach could only be roughly approximated.

We are certain that the pouch of the entire stomach uniformly stimulated with histamine provides the most accurate assay data. Hence, we shall not present all our data obtained from Pavlov pouch dogs, but shall restrict the data to that showing the comparative effects of the enterogastrone and olive oil.

TABLE 7

Effect of enterogastrone and olive oil on response of Pavlov pouch dog to a meal

	MEAL CONTROL		1 UNIT OF ENTEROGASTRONE				50 CC. OF OLIVE OIL			
	Volume	HCl	Volume	Inhibition	HCl	Inhibition	Volume	Inhibition	HCl	Inhibition
	cc.	*mgm.*		*per cent*	*mgm.*	*per cent*		*per cent*	*mgm.*	*per cent*
1st hour	28.8	132.5	12.9	−55	51.2	61	4.3	−85	12.3	−91
2nd hour	20.1	100.9	17.5	−13	88.8	12	7.0	−65	21.9	−78
3rd hour	14.5	72.9	13.0	−13	65.4	10	7.0	−52	27.0	−63
4th hour	8.0	36.2	9.0	+13	41.0	+13	10.4	+30	42.4	+17
5th hour	5.5	17.8	2.1	−7	18.9	+6	14.5	+164	63.6	+257
Total.....	76.9	360.3	57.5	−25	265.3	−38	43.2	−44	167.2	−54

In table 7 are shown the comparative secretory responses (average of 3 tests each) of a Pavlov pouch dog fed the test meal alone, with one unit (intravenously) of enterogastrone, or with 50 cc. of olive oil. It will be noted that the enterogastrone exerts its greatest effect during the first hour (61 per cent inhibition), but its action persists in a diminishing degree for three hours. In the fourth hour there is a slight increase over the control, presumably because the intestinal phase of gastric secretion has been delayed as a result of the slowed gastric evacuation after enterogastrone. Olive oil has a greater effect than one unit of enterogastrone (91 per cent for the first hour) and its effect lasts for four hours. However, after this period the marked excitatory effect of digested fat products becomes prominent.

As will be noted in table 8, fat is only very slightly effective (12 per cent inhibition) in depressing the secretion stimulated by a single subcutaneous dose (0.1 mgm. per kilo) of histamine. This is probably due to the fact that the histamine in addition to being a more potent stimulus than the

meal, reached its maximum effect much earlier. Also, one unit of enterogastrone (27 per cent inhibition), although more effective than olive oil, does not manifest the degree of inhibition which it manifested against a meal (61 per cent). This again is probably attributable to the fact that histamine administered in a single dose is a more potent stimulus than a meal during a period of one hour.

Another point that should be noted is that, although only 27 per cent inhibition was obtained when the histamine was given in a single dose, 62 per cent inhibition was obtained when the histamine was given "continuously" (repeated small doses) in the same animal. In the latter experiment, 0.05 mgm. histamine was injected every ten minutes (0.3 mgm. per hour) and resulted in the secretion of 21 cc. of juice per hour, which was the same as that secreted in response to a single dose of 1.4 mgm. histamine. This was also estimated to represent 1 cc. of juice per

TABLE 8

Effect of enterogastrone and olive oil on response of Pavlov pouch dog to histamine

	CONTROL		ONE UNIT ENTEROGASTRONE				50 CC. OF OLIVE OIL			
	Volume	HCl	Volume	Inhibition	HCl	Inhibition	Volume	Inhibition	HCl	Inhibition
		mgm.		*per cent*	*mgm.*	*per cent*		*per cent*	*mgm.*	*per cent*
15 minutes	2.7	6.81	1.8	−33	2.74	−60	2.4	−11	5.02	−26
30 minutes	9.9	41.60	8.0	−19	29.70	−29	8.7	−12	32.10	−23
45 minutes	6.4	30.10	5.6	−13	25.40	−19	6.8	+8	30.30	+1
60 minutes	2.1	8.41	1.5	−29	6.08	−28	2.1	0	8.80	+6
Total.....	21.1	86.92	16.9	−20	63.92	−27	20.0	−5	76.92	−12

minute from the whole stomach. This ineffectiveness of enterogastrone against a single large dose, as compared with a "continuously" administered small dose of histamine, is due to the fact that the single dose is a more potent stimulus, during a portion of the hour, than the smaller dose given at intervals. When 50 cc. of olive oil were given orally with the "continuous" administration of histamine, the secretion of hydrochloric acid was inhibited to the extent of 45 per cent instead of 12 per cent, which supports the foregoing interpretation.

The results obtained after administering four units of enterogastrone *subcutaneously* to a Pavlov pouch dog fed the test meal are shown in table 9. Here the inhibitory effect is marked, amounting to 51 per cent for the first hour and lasting four hours. This result differs from that obtained on pouches of the entire stomach receiving "continuous" histamine, which may be due to the meal being a less potent stimulus than histamine.

Activity when administered orally? When active preparations of entero-

gastrone were given orally or introduced directly into the duodenum, inhibition of motility frequently resulted; but we could not be certain that the inhibition was specific, since a similar inhibition was sometimes obtained by the administration of inactive control preparations or substances. When ten and twenty units were given orally to dogs with a pouch of the entire stomach stimulated "continuously" with histamine, inhibition of secretion was never observed.

Other physiological effects. Assays have shown that the tannic acid preparations in less than 0.5 mgm. doses will stimulate the secretion of ten drops of pancreatic juice in ten minutes. This indicates that practically all the secretin contained in the original "secretin A precipitate" is retained in the tannic acid material. Although assays for cholecystokinin have not been performed, in all probability it is also present in significant quantities.

TABLE 9

Effect of subcutaneous enterogastrone on response of Pavlov dog to a meal

TIME	CONTROL MEAL		4 UNITS ENTEROGASTRONE SUBCUTANEOUSLY			
	Volume	HCl	Volume	Inhibition	HCl	Inhibition
		mgm.		*per cent*	*mgm.*	*per cent*
1st hour	31.7	140.8	17.3	−45	68.1	−51
2nd hour	25.9	130.8	19.2	−26	87.4	−33
3rd hour	19.5	93.3	14.9	−24	72.3	−23
4th hour	13.9	66.1	12.6	−9	57.7	−13
5th hour	6.6	27.6	9.6	+45	42.9	+55
Total.....	97.6	458.6	73.6	−25	327.4	−29

The tannic acid preparations are not completely free of vaso-depressin. One hundred milligrams (two units) produce a fall in blood pressure of about ten millimeters of mercury.

Discussion. Certain significant differences were noted in the response of the various types of animals employed in this work to enterogastrone and olive oil.

One unit of enterogastrone administered intravenously to "motility" dogs inhibited gastric contractions for approximately one hour, but when injected into Pavlov pouch dogs receiving a meal or into pouch dogs receiving "continuous" histamine the effect is evident for from 3 to 4 hours. Lim and his co-workers (5), (6) found that enterogastrone inhibited secretion in their Heidenhain pouch dogs for 3 hours, but acted on an average of only 30 minutes when administered to dogs showing hunger contractions.

Another marked difference between the effect of enterogastrone on the

motor and secretory functions of the stomach is that small doses completely inhibit motility for a period proportional to the dose, whereas such doses have no effect whatsoever on gastric secretion in total pouch dogs receiving "continuous" histamine. Furthermore, doses which are effective under the latter conditions do not inhibit secretion completely for a period followed by gradual recovery, but instead merely lower the secretory rate to a constant, reduced level which persists for several hours. Apparently it is characteristic of enterogastrone to have a somewhat different type of action on the two types of functional activity of the stomach.

Certain discrepancies were noted in the subcutaneous action of enterogastrone. Four units administered subcutaneously are able to inhibit gastric motility for approximately one hour. The same dose is also effective against the secretion stimulated by a meal in Pavlov pouch dogs. In total pouch dogs receiving "continuous" histamine, however, the tannic acid preparations have invariably produced a slight stimulation of secretion. Assays of these preparations on blood pressure have shown 100 mgm. or two units to exhibit some depressor effect. In all probability this vasodilator substance consists largely of histamine which would account for its stimulatory properties on subcutaneous but not on intravenous injection. That this impurity should not interfere with the inhibitory action on gastric motility on subcutaneous administration is indicated by the work of Ivy and Vloedmann (20) who found histamine to be without effect on gastric motility when given subcutaneously in doses capable of producing marked stimulation of secretion. However, the tannic acid preparations retain their inhibitory power when injected subcutaneously in Pavlov pouch dogs fed a meal. This may be attributed to the fact that the small quantity of contaminating histamine is a subthreshold dose, which can be effective only when the gastric glands are already responding to histamine, and also to the fact that the meal secretion is more readily inhibited than histamine secretion.

The results obtained with olive oil indicate that it is much more potent when administered to Pavlov pouch dogs than to total pouch dogs, even under comparable conditions of "continuous" histamine stimulation. It was occasionally observed, when olive oil was given by tube to the total pouch dogs, that in about one hour the oil had traversed the entire gastro-intestinal tract and was leaking from the rectum. It is probable that the olive oil did not remain in contact with the duodenal mucosa for a period sufficiently long to elicit its normal degree of inhibition. This condition does not exist in the Pavlov pouch dogs because the oil is retained in the main stomach and is eliminated gradually into the duodenum.

Another important finding is the difference in the ability of enterogastrone to inhibit secretion in response to a meal and to histamine. One

unit of enterogastrone administered intravenously to Pavlov pouch dogs receiving a meal was able to inhibit the production of acid 40 per cent during the first two hours. When administered to the same dog receiving 1.4 mgm. histamine subcutaneously in a single dose, the secretion was inhibited only 27 per cent, in spite of the fact that the histamine produced much less secretion during the first hour than the meal. Lim and his co-workers (5) similarly observed 71 per cent inhibition of the meal secretion in their Heidenhain pouch dogs as compared with only 48 per cent inhibition against a single dose of histamine. The fact that enterogastrone is more effective against the secretion stimulated by a meal than against that resulting from a single dose of histamine is due, we believe, to a quantitative difference in the character of the two stimuli rather than to a qualitative one. A lengthy discussion of our histamine data and of the literature would be required to substantiate this point.

Is one chemical substance responsible for both motor and secretory effects? Lim (9) has reviewed his evidence regarding the motor and secretory effects of enterogastrone and concludes that they are both properties of a single substance. The potency of his preparation made from various tissues in inhibiting gastric secretion paralleled their potency in affecting hunger contractions. This we can confirm. There is no evidence to indicate that two agents are involved. However, conclusive proof that only one agent is responsible for both effects awaits the chemical isolation of the active principle or principles.

CONCLUSIONS

1. A method is described for the preparation of an extract (enterogastrone) from the duodenal mucosa of hogs, which exhibits an inhibitory action on gastric motility and secretion. The inhibition has all the characteristics of being specific.

2. An accurate method for the biological assay of such extracts is described, and a tentative unit of potency defined.

3. The inhibitory effect of the extract on hunger contractions and gastric motility initiated by distention was found to be the same and the duration of the effect was shown to be related to the dose.

4. The extract was shown to be effective in inhibiting the secretory response of dogs with pouches of the stomach to a meal and to histamine administered "continuously" (a small dose every ten minutes) or in a single dose.

5. The inhibitory effect of the extract on gastric secretion maintained at a uniform rate by the repeated injection of a small dose of histamine, was found to increase with the size of the dose of enterogastrone and to vary oppositely to the strength of the secretory stimulus, within the limits of the dosage and stimuli used. The secretory response to a meal

of meat is more readily inhibited than the secretory response to a single dose of histamine.

6. The extracts administered subcutaneously are effective in four times the intravenous dosage. Conclusive evidence for activity by the oral route has not been obtained.

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VITAMIN A DEFICIENCY IN THE DOG

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Seven dogs, 1 year old, were used in this experiment. Since birth they had been maintained on a general diet. Of the seven, six were litter mates. At the beginning of the experiment, one of the dogs was killed and the vitamin A content of the liver determined. Of the remaining six dogs, two controls were fed on a vitamin A-deficient diet[1] plus 7.2 grams of cod liver oil[2] weekly (1800 I.U. vitamin A and 175 I.U. D per gram). The other four received only the vitamin A-deficient diet. One of the four on the vitamin A-deficient diet had two litters of puppies. They were fed their mother's milk and the A-deficient diet. Complete blood cell studies were made, and weights were taken bi-weekly or monthly on all dogs. The vitamin A content of all livers was determined at the end of 52 weeks.

Preparation of diet. The vitamin A-deficient diet (Massengale, 1935) used in this experiment was made in cakes of convenient size and baked. The deficiency of the diet was verified periodically by feeding the same to standard 21 to 28 day old rats. In each instance the rats showed a neutrophilic index lag and either a weight loss or xerophthalmia, or both, within 28 to 35 days.

Method of determining vitamin A content of liver. The total liver weight was noted. To each 100 grams of finely ground liver 500 cc. of 5 per cent potassium hydroxide were added. After standing 48 hours this mixture was extracted eight times with ether and the extracts washed three times with distilled water, or until the washings were neutral to litmus; the water

[1] Diet:

	per cent
A-deficient mixture (consisting of 35 parts whole wheat; 35 parts white maize; 20 parts ground meat; 5 parts crude casein; all extracted with acetone 36 hours and dried in vacuum oven at 100°C. for 12 hours)	80
Brewer's yeast	10
Maize oil (containing irradiated ergosterol, 5 I.U. per gram of oil)	7
Bone meal	2
NaCl	1

Analysis: Carbohydrates, 56 per cent; protein, 24 per cent; fat, 9 per cent; minerals (ash) 5 per cent; crude fiber, 1 per cent; moisture, 5 per cent.

[2] Purchased from Mead Johnson and Company.

was removed by adding anhydrous sodium sulphate. The extract was then filtered and evaporated under vacuum to dryness and weighed. Spectrophotometric determinations of vitamin A in the residue were made with the Hilger Vitameter.

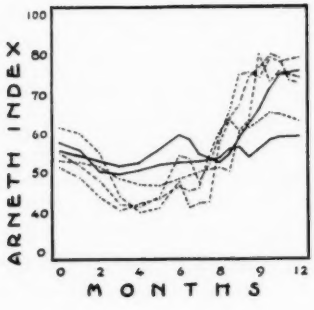

Fig. 1

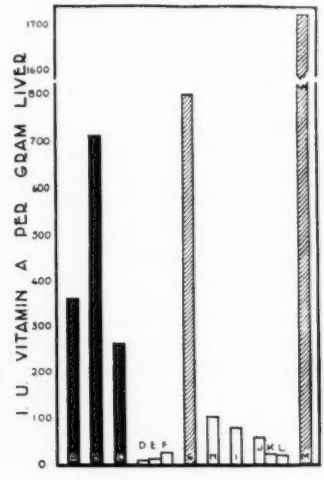

Fig. 2

Fig. 1. Arneth indices of dogs in avitaminosis A (interrupted lines) and controls (continuous lines) over a period of one year.

Fig. 2. Vitamin A international unit content per gram of liver.

A. Normal—general diet.

B.
C. } Controls—A-deficient diet plus 7.2 grams cod liver oil per week, 52 weeks.

D.
E. } A-deficient diet for 52 weeks.
F.

G. A-deficient adult for 52 weeks followed by 2,400,000 I.U. of vitamin A.

H. Newborn puppies (mother on general diet).

I. Puppies—A-deficient 1 month—nursed by A-deficient mother.

J. Pup—A-deficient 3 months—nursed by A-deficient mother.

K. Same as J.—litter mate.

L. Same as J.—separate litter.

M. A-deficient pup for 3 months, followed by 1,200,000 I.U. of vitamin A.

Neutrophil maturation lag in avitaminosis A in the dog. In a previous study (Crimm and Short, 1935a) on the rat it was found that the neutrophil index lag was an early manifestation of the cellular changes associated with vitamin A-deficiency. In this series of A-deficient dogs a neutrophil index lag became definitely manifest at three months and persisted to six months (fig. 1). There was a gradual increase in the gross

white counts, while the Arneth (1904) indices tended to lag. This indicates a degenerative shift in the granulocytes in A-avitaminosis.

The controls (dogs B and C, fig. 1) failed to evince a similar neutrophil lag, but their Arneth index continued on essentially a horizontal gradient, with the exception of one control which had a mild, chronic respiratory infection from the fifth to twelfth months. After eight months on a vitamin A-deficient diet the dogs showed a definite "left shift" of the Arneth index (fig. 1) and in the Schilling count, with gross leucocytosis as is found with infection, the result of prolonged vitamin A-deficiency. Presumably, the absence of vitamin A inhibited the normal formation of granulocytic

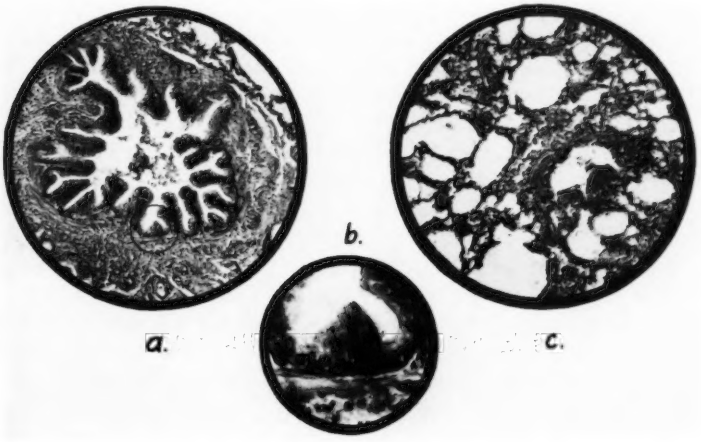

Fig. 3. a. Photomicrograph (× 50) of bronchiolar epithelium showing early cellular metaplasia.

b. Detail of above (× 225).

c. Photomicrograph (× 50) showing pneumonitis of prolonged avitaminosis A, with metaplastic cell nests.

cells and caused a "right shift" until infection set in at the eighth month. This qualitative granulocytic change was the only evidence of vitamin A-deficiency observed *ante mortem* in the dogs in these experiments. Xerophthalmia and progressive weight loss were not observed in any instance. However, early metaplasia of the bronchiolar epithelium was manifest at autopsy, together with a moderate pneumonitis (fig. 3).

General blood picture in avitaminosis A in the dog. The hemoglobin remained at a higher level in the control animals, except during the first two months. After four months, deficient animals had lower hemoglobin levels. The red blood cell count essentially paralleled the hemoglobin.

The anemia of the vitamin A-deficient animals simulates the nutritional type. The gross leucocyte count varied considerably in both the normal and deficient dogs, but the vitamin A-deficient animals averaged higher white counts than did the control animals.

In dogs G and M (fig. 2) which had a single, large dose of vitamin A following a prolonged deficiency, a "left shift" of the granulocytes occurred. The total white count remained essentially unchanged. The hemoglobin decreased and the red blood cell count increased. This qualitative "left shift" of the granulocytes has been previously observed in normal human individuals following the administration of vitamin A as halibut liver oil over prolonged periods (Crimm and Short, 1935b).

Vitamin A content in the liver of dogs. The vitamin A content in the liver of dog A (fig. 2) was determined at the beginning of this experiment while all dogs were on general diet. Spectrophotometric analysis of this dog's liver fat showed 360 units of vitamin A per gram of liver. The two controls (dogs B and C), which were fed 7.2 grams of cod liver oil weekly for 52 weeks, had 710 and 260 units of vitamin A per gram of liver respectively. The vitamin A-deficient dogs (dogs D, E and F) after 52 weeks had 10, 14 and 26 I.U. of vitamin A per gram of liver respectively.

Following 52 weeks of vitamin A-deficiency, dog G (fig. 2) was given 2,800,000 units of vitamin A at a single feeding. After 10 days the dog was killed and the vitamin A content of the liver found to be 800 units of vitamin A per gram, or a total vitamin A liver content of 332,000 I.U. This shows that a large quantity of vitamin A can be assimilated and stored. A three months' old deficient puppy (dog M) was given 1,400,000 I.U. of vitamin A at a single feeding and the liver content was determined 10 days later. The vitamin A content was found to be 1700 I.U. per gram of liver, or a total vitamin A liver content of 210,000 I.U. Both dog G and dog M were practically A-deficient when given the large dose (table 1). Thus, a three months' old puppy (dog M, table 1, fig. 2) absorbed and assimilated about 40 times the amount of vitamin A per kilogram of body weight as did the adult animal.

The livers of three newborn puppies, from a mother on a general diet, were found to contain 100 units of vitamin A per gram (fig. 2, H). One of the vitamin A-deficient bitches had five puppies. After nursing the deficient mother for one month, the puppies were killed, and the pooled livers (fig. 2, I) were found to contain 80 I.U. of vitamin A per gram. The other two puppies (dogs J and K) were continued on the vitamin A-deficient diet for three months. Their liver content was 65 and 25 I.U. of vitamin A per gram respectively. The same vitamin A-deficient dog had a second litter of puppies, after having been on a vitamin A-deficient diet for nine months. One puppy (dog L) was fed its mother's milk and a

vitamin A-deficient diet for three months. The liver assayed 23 I.U. of vitamin A per gram.

Utilization of vitamin A in the dog. Two litter mates (dogs B and C) were given a vitamin A-deficient diet, plus a total of 93,600 I.U. of vitamin A, over a period of 52 weeks. The livers contained, on the average, a total of 124,000 I.U. of vitamin A, or 9,200 I.U. per kilogram of body

TABLE 1

Vitamin A content of dog livers

	EXPERIMENT	LIVER WEIGHT	I.U. VITAMIN A PER GRAM LIVER	TOTAL UNITS	GROSS BODY WEIGHT	I.U. PER KGM. BODY WEIGHT, AVERAGE
		grams			*kgm.*	
Normal	A	330	360	119,000	13.9	8,550
Controls*	B	225	710	160,000	12.8	9,200
	C	335	260	87,000	14.4	
	Average			124,000	13.5	
A—Deficient	D	285	26	7,400	14.7	
	E	235	14	3,300	9.9	400
	F	256	10	2,600	9.1	
	Average			4,400	11.2	
Normal puppies†	H (Newborn)	30	100	3,000	0.8	3,800
A—Deficient puppies	I (1 month pups)	38	80	3,000	1.0	2,900
	J (92 days)	130	65	8,400	3.6	2,330
	K (92 days)	185	25	4,400	4.4	1,000
	L (92 days)	165	23	3,800	3.5	1,080
A—Hypervitaminosis	G (52 weeks deficient adult)	415	800	332,000	13.4	2,480
	M (92 days old deficient pup)	123	1,700	210,000	2.2	96,300

* 1800 I.U. vitamin A and 175 I.U. D per gram per week = 93,600 I.U. of vitamin A (total amount administered).

† From mother on a general diet.

weight. Meanwhile dogs D, E and F, maintained 52 weeks on the vitamin A-deficient diet, averaged 4,400 I.U. of vitamin A for the total liver content, or 400 I.U. per kilogram of body weight. The dog sacrificed before the others were placed on the above diets had 8,550 I.U. per kilogram of body weight (total 119,000 I.U.).

If only the vitamin A stored at the beginning of the experiment were available in dogs D, E and F, it would appear by difference from dog A

(normal) that about 157 I.U. of vitamin A per kilogram of body weight per week were utilized. The amount of vitamin A recovered from the liver, following administration of 93,600 I.U. over a period of one year, is about 750 I.U. per kilogram of body weight, or about 44 per cent of that amount given (by difference between dogs B, C and A). If 44 per cent of the vitamin A given dogs B and C were absorbed, it would appear by difference between dogs D, E and F that the rate of utilization is 330 I.U. per kilogram of body weight. It is, therefore, apparent that the vitamin A requirement in the dog is between 157 and 330 I.U. per kilogram of body weight per week. If the requirement of vitamin A for man corresponds to that of the dog, it would seem that the daily requirement of vitamin A for a man weighing 60 kgm. would be 1800 I.U. or approximately 2 grams of cod liver oil.

SUMMARY

1. In these experiments on vitamin A-deficiency in dogs, rats were used to confirm the A-deficiency of the diet. Evidence of the A-deficiency was shown by a neutrophil index lag, weight loss and xerophthalmia.

2. A neutrophil index lag (modified Arneth index) was found to be the earliest manifestation of the cellular changes associated with vitamin A-deficiency in the dog.

3. Xerophthalmia and loss of weight were not observed in dogs which received a vitamin A-deficient diet for one year. Early metaplasia of the bronchiolar epithelium, with pneumonitis, furnished evidence of the A-deficiency. Spectrophotometric assay of the livers showed essential depletion.

4. Of the vitamin A given in the form of cod liver oil, 44 per cent was recovered from the liver.

5. Following the administration of a massive dose of vitamin A to A-deficient dogs, a three months' old puppy absorbed and assimilated in the liver about 40 times as much vitamin A as did an adult dog.

6. The vitamin A utilization rate in the dog is between 157 and 330 I.U. per kilogram of body weight per week.

The authors gratefully acknowledge the valued assistance of Miss Grace L. Heimann, M.T.

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EXTRAPYRAMIDAL CONTROL OF MICTURITION: THE EFFECTS OF LESIONS OF THE TEGMENTUM OF THE MID-BRAIN[1]

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In previous reports we have shown the influence of cortical and subcortical mechanisms in the cerebral control of the smooth muscle of the bladder. Removal of the cerebral motor cortices causes an immediate decrease in vesical capacity (Langworthy and Kolb, 1933); the capacity then gradually increases but does not regain the normal size (Langworthy and Hesser, 1936). Electrical stimulation of portions of the motor cortex causes, after a latent period, a rise in vesical pressure initiating micturition (Langworthy and Kolb, 1935); when the pressure is high, cortical stimulation produces a fall of pressure. The fiber tract subserving this cortical influence on micturition may be followed through the internal capsule, cerebral peduncle and pyramid.

A distinct subcortical mechanism is evident from the further decrease in vesical capacity by decerebration of the decorticate preparation and from the responses to electrical stimulation of transverse sections of the brain stem. Stimulation of areas in the periventricular gray substance of the mid-brain, pons and medulla oblongata causes abrupt strong contractions of the vesical muscle. The response is not affected by previous decortication and is therefore of subcortical extrapyramidal origin. Barrington (1925) placed lesions injuring this pathway and produced frequent micturition. Recently Kabat, Magoun and Ranson (1936) have stimulated this region with the Horsley-Clarke stereotaxic instrument. They obtained bladder contraction, dilatation of the pupils, marked rise in blood pressure, increased rate and depth of respiration, and frequently struggling movements. These various responses usually appeared together but could be dissociated. The pathway could be traced forward into the lateral hypothalamus.

In the present paper experiments are described which illustrate the acute and chronic effects of lesions of this extrapyramidal pathway.

METHOD. Seventeen female cats were used in the experiments. Nem-

[1] This study of the control of the bladder by the central nervous system was supported by a grant from the National Research Council.

butal, administered intraperitoneally, provided anesthesia for the operation and subsequent cystometric study. Readings of the vesical function, by the air-water system previously described, were made before operation, immediately after and then at intervals of several days.

The operative lesions were made in the following manner. With aseptic precautions an opening was made in the skull in the subtemporal region, and the dura mater was opened. The mid-brain was exposed by retracting the temporal lobe. With a fine knife a transverse lesion was placed across the tegmentum of the mid-brain, avoiding the cerebral peduncles and extending into the central gray matter, being limited dorsally by the anterior margin of the tentorium. The lesion was usually made caudal to the oculomotor nuclei. Bleeding was readily controlled and little change in the animals' condition was observed during the procedure. The extent of the lesion was determined by postmortem examination of every case.

SYNDROME OF THE MESENCEPHALIC TEGMENTUM IN THE CAT. Characteristic changes in both striated and smooth muscle were observed in the cats following the operation. We may best present the syndrome in two stages, the acute effects, some of which were evident before the anesthesia had worn off and which lasted several days, and the later symptoms, which were fully developed after a week.

Acute stage. For the first few days after operation it was difficult to ascertain whether the animals were conscious. The corneal reflexes were preserved, but there was a profound inactivity of the somatic musculature. There was little response to external stimuli, except for an occasional withdrawal movement. The head could not be held up without support. The extremities were usually somewhat flexed. Often the trunk was twisted so that the head and chest faced upwards and the fore-legs tended to be held off the floor. There was a decreased resistance of the legs to manipulation. The animals had to be fed by stomach tube.

The disturbances in the autonomic sphere were equally striking. The pupils were constricted to a width of less than 0.5 mm., appearing as narrow slits. They dilated well upon the instillation of a solution of cocaine into the conjunctival sac. A few times there was mydriasis from a lesion of the oculomotor nucleus or nerve. The bladder usually emptied in the course of the operation, during the exposure or section of the mid-brain. For several days thereafter, micturition and defecation were frequent and forceful. An increased resistance was felt in the esophagus on passing the stomach tube. In several cases the action of the heart was irregular, due to frequent extrasystoles.

This syndrome occurred in 15 of the 17 cats, in 10 of which it was fully developed. In the remaining two the entire picture was lacking; the pupils and bladder were quite unaffected and the animal was able to

walk on the day after the operation. Fairly extensive lesions in the tegmentum were made in these animals, but they did not extend as far dorsally as in the other experiments.

Later stage. In a week the picture gradually changed. The animals began to move about, first with slight rhythmic movements of the extremities. Later they could often hold their heads up, sit and even perhaps take a few steps. In the animal at rest, the attitude was one of flexion of all joints of the extremities, the claws being often uncovered. The head was retracted. There was a generalized rigidity of such an extent that the animal maintained the posture of its parts when it was lifted. A suggestion of catalepsy was present in some cases. On manipulation of the legs it was found that the rigidity was present in both flexor and extensor muscles. Furthermore, the resistance to manipulation did not lessen during the full extent of lengthening and shortening. This nonselectivity of hypertonus and the absence of a lengthening reaction presented a striking similarity to the lead-pipe rigidity found in patients with the parkinsonian syndrome. The rigidity was not due to irritation from a meningitis or peritonitis. In one preparation, reflex extension of the contralateral hind-leg and both fore-legs resulted from stimulation of the foot pad of one hind-leg.

The pupils after 3 days slowly widened, and after a week were 1 to 2 mm. in width. Nystagmus was observed in a few cases. Micturition and defecation became less frequent and the heart rate regular. In one instance an interesting spasm of the respiratory muscles was observed. Clonic movements of the diaphragm occurred during expiration, with slight associated contractions of the epiglottis and palate. As many as three such ties occurred with each expiration.

Analysis of changes in the urinary bladder. The striking alteration in vesical function is readily subject to analysis by cystometric examination. The findings are discussed in terms of typical examples.

Experiment 1. The normal reading, taken immediately prior to the operation, revealed a vesical capacity of 35 cc. (fig. 1A). Fluid escaped from the urethra at a pressure of 35 cm. of water, in the absence of an active emptying contraction of the bladder. The pressure in the empty bladder was 4 cm.; after 20 cc. of fluid were added it was 7 cm., and during the remainder of the filling it was high.

Immediately after section of the tegmentum of the mid-brain, a distinct change appeared in the reaction of the bladder muscle to filling (fig. 1B). Following the introduction of the first 5 cc. of water, the pressure was slow in falling to a resting level and fluid escaped at 16 cm. pressure. Thereafter periodic waves of contraction appeared, at intervals of 20 to 30 seconds. They often raised the pressure to 16 cm., when fluid would escape from the urethra. The waves showed some tendency to summation

and at times the pressure remained high for a considerable time. Addition of a second 5 cc. of fluid caused a similar reaction, the resting pressure now being somewhat higher, about 7 cm. of water.

The influence of peripheral stimuli in the sacral region was now tested (fig. 1B). Rhythmic pinching of the tail or dilatation of the vaginal orifice with a clamp caused stronger vesical contractions and the escape of a considerable amount of fluid from the urethra. The vesical muscle was thus unusually irritable to peripheral stimuli.

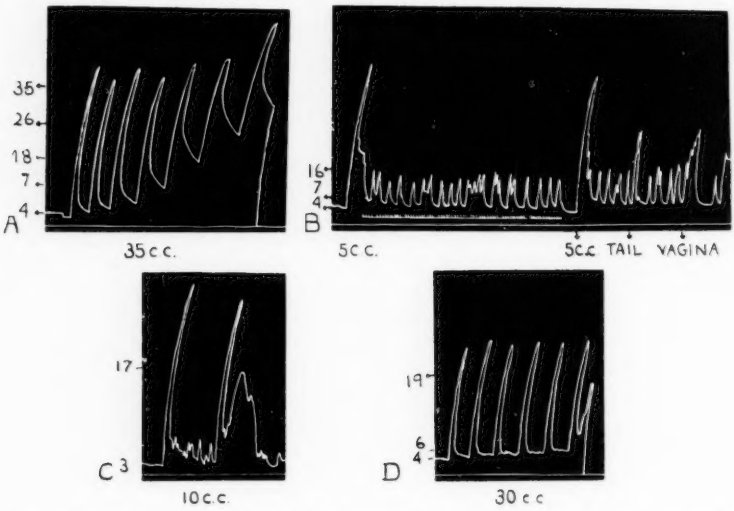

Fig. 1. Experiment 1. Series of bladder readings showing the characteristic effect of the tegmental lesion on vesical activity. The vertical rises of pressure spaced at fairly equal intervals indicate the points at which 5 cc. of fluid were added to the system. A, normal. B, immediately following the operation. At the points marked *tail* and *vagina* these structures were mechanically stimulated. Time is recorded at 5 second intervals. C, 3 days later. D, 6 days later.

Another reading (fig. 1C) was made 3 days after operation. After the addition of the first 5 cc. of fluid, the pressure fell quickly and waves of small amplitude appeared. When a second quantity of fluid was added the pressure fell slightly; then a contraction wave occurred, raising the pressure to 17 cm. with the escape of fluid. This pressure was maintained until the bladder emptied completely. Such spontaneous contractions sufficient to empty the bladder are not seen in the normal, deeply anesthetized cat. One may infer, therefore, that the stretch reflex was hyperactive.

The final reading (fig. 1D) was made 6 days after section of the mid-

brain. Very small waves were present during filling. The vesical capacity had now increased to 30 cc. The pressure remained fairly low until filling was complete, when a contraction wave raised the pressure to 19 cm. and emptying occurred. This strong contraction of the bladder wall indicated that the stretch response was still hyperactive.

Experiment 2. Before operation, the bladder held 75 cc. of fluid and did not empty at a pressure of 35 cm. (fig. 2A). The resting intravesical

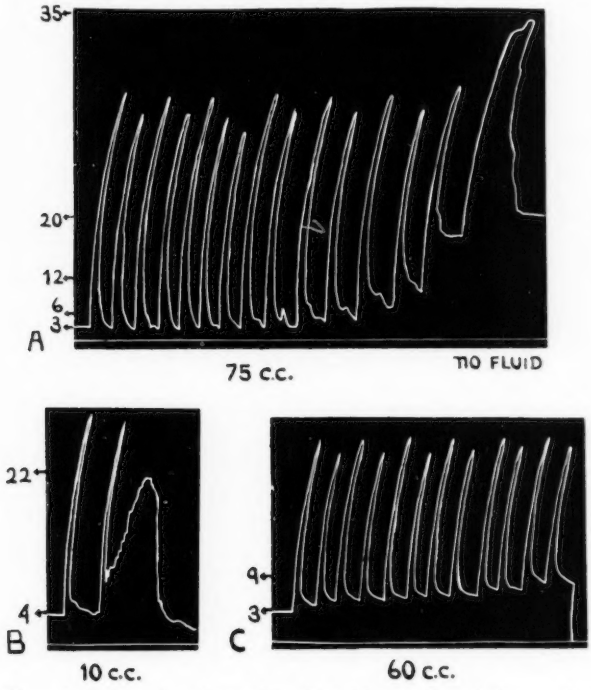

Fig. 2. Experiment 2. Another series of bladder readings before and after operation on the mid-brain. A, normal. B, immediately after the operation. C, 10 days later.

pressure remained low until filling was nearly complete. A normal stretch response may be seen as quantities of fluid were added.

The immediate post-operative record (fig. 2B) showed a normal response to the addition of 5 cc. of fluid, with a prompt fall and the absence of spontaneous waves. Another 5 cc. increment was followed by a fall in pressure, which then rose with a series of contractions to 17 cm. with emptying. The vesical capacity thus had lessened from 75 cc. to 10 cc. with the development of a hyperactive stretch response.

Ten days after the operation, another record (fig. 2C) showed an incomplete return of the bladder volume towards normal, now being 60 cc. The pressure at which the bladder emptied, 9 cm., was considerably lower than the previous emptying pressure. The pressure remained low during filling and no spontaneous waves of contraction occurred.

This cat resembled the first one described in the increased response of the vesical muscle to stretch, and differed from it in the absence of spontaneous rhythmic activity of that muscle. It was particularly noteworthy because of the full development of the syndrome of the mesencephalic tegmentum, as described above.

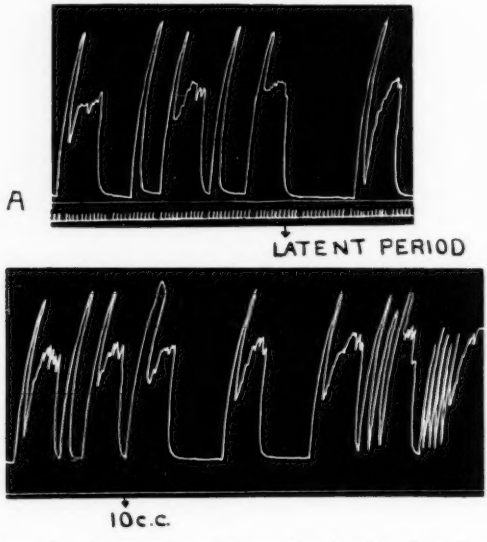

Fig. 3. Experiment 3. A, record made immediately after the lesion was made in the mid-brain. Time is recorded at 5 second intervals. B, continuation of same. All increments of fluid were in 5 cc. quantities except for the one noted as 10 cc., and the series of 2 cc. increments at the right.

Experiment 3. This experiment will not be cited in detail as it was similar to those already described. The feature we wish to present appeared in the record obtained immediately after the operation upon the mid-brain (fig. 3, A and B). Following the addition of the first 5 cc. of fluid a strong stretch response formed by a summation of waves maintained the pressure high for 35 seconds; the pressure then fell to a normal resting level. The addition of a second increment induced no stretch response but the reflex contractions followed the third increment. It was found repeatedly that stretch responses were produced by the addition of every other quantity

of fluid. Similar observations have frequently been noted in the study of the bladder of patients with lesions of the nervous system caudal to the mid-brain (Langworthy, Lewis, Dees and Hesser, 1936).

That this was dependent upon the existence of a refractory period may be judged from the records. With the first 5 increments of fluid, stretch responses appeared after only the first, third and fifth ones. The interval between fillings was then prolonged to 2 minutes and 20 seconds. The next increment was promptly followed by a summated series of contraction waves. This demonstrated that volume alone is not the deciding factor in the production of the hyperactive stretch response. By varying the intervals between increments, it could be determined that one and a half minutes after a series of contraction waves, the addition of 5 cc. of fluid was not adequate for the repetition of this type of response. After longer intervals, the 5 cc. increment was regularly followed by vigorous contraction waves.

The refractory period of the bladder wall was relative, for a larger increment (10 cc.) would induce at this time a stretch response. This is shown in the middle of graph B (fig. 3). However, when such a large increment was divided into 5 cc. portions and the bladder was permitted barely to adapt itself to each portion, a still larger amount of fluid (15 cc.) could be added before a vesical contraction occurred, as is shown in the final portion of graph B. When fluid was then added in frequent 2 cc. lots, a total of 10 cc. again sufficed for an emptying contraction of the bladder.

Discussion. Injury to the tegmentum of the mid-brain in the cat caused profound and widespread disturbances in somatic and visceral motor function. Striated musculature showed at first decreased tone and later a peculiar hypertonus which was not unlike the parkinsonian rigidity of man. Smooth muscle structures (circular muscle of iris, bladder, rectum and esophagus) were strongly contracted immediately after operation, with a slow recovery beginning after several days. All the changes went hand-in-hand, and when partial effects were produced in one structure, those in others were equally incomplete. In the few instances that the animals were able to walk the day after operation, the pupils were invariably not miotic and the bladder not contracted.

These effects were due to the lesion of the mesencephalic tegmentum. The cerebral peduncles were spared. Furthermore the physiological disturbances were quite different from those caused by lesions of the motor cortex and its projection fibers. In the experiments which failed to produce the tegmental syndrome, the lesions differed from those in the successful cases in that they did not extend as far dorsally. They spared the areas ventrolateral to the aqueduct of Sylvius which on electrical stimulation gives a contraction of the vesical muscle. Inasmuch as the entire

somatic and visceral syndrome is evidently produced by the lesion of this relatively small area, it would seem that extrapyramidal fiber-tracts of tremendous importance in the economy of the entire animal pass through this portion of the mid-brain. The origin of this pathway remains to be determined.

The changes in the urinary bladder are, of all, most accessible to physiological analysis by means of graphic records. There was decrease in the capacity of the bladder, associated with hyperexcitability of the detrusor to reflex stimulation. Such heightened contraction of the vesical muscle could be elicited by stretch stimuli and by peripheral stimulation in the sensory domain of the sacral and coccygeal segments (fig. 1B). This suggests an increased irritability of the vesical reflexes in the spinal cord, as a result of the removal of an extrapyramidal inhibitory influence.

In the experiments described above, the effect on the pressure at which the vesical sphincters relaxed should perhaps not be compared, since in the pre-operative studies under deep anesthesia bladder emptying is usually a passive process. In two experiments, performed under lighter anesthesia, emptying contractions of the bladder occurred before operation as well as after. In these cases there was no definite change in the pressure at which the sphincters relaxed. In experiment 2, cited above, a decrease in this pressure occurred during the post-operative period.

Spontaneous rhythmic waves of vesical contraction sometimes were seen immediately following operation, as shown in figure 1B. Similar waves were sometimes observed after removal of the cerebral motor cortices (Langworthy and Hesser, 1936), but they disappeared within a few hours. After an operation upon the tegmentum, the waves persisted for several days, gradually decreasing in amplitude.

The changes seen immediately after the tegmental cut bear other similarities to those occurring after removal of the motor cortices. After both procedures the stretch reflex becomes hyperactive, with a resulting decrease of the bladder volume. There is one difference in the nature of the vesical contraction produced by stretch. After the tegmental lesion, this response is clearly due to summation of a series of contractions of the bladder muscle. Following bilateral motor decortication the response to stretch consists rather of a single strong contraction of the detrusor, produced by better fusion of vesical contraction waves. Finally the tegmental syndrome may be produced in cats from which both motor cortices have previously been removed. Details of these experiments will be described later. The effects on the pupils and somatic musculature are, of course, quite different after the two procedures.

SUMMARY

Injury to the tegmentum of the mid-brain in cats causes a syndrome consisting of severe motor disturbances of both striated and smooth

muscle. There is profound inactivity of the body which changes into a state resembling the parkinsonian syndrome of man. The pupils become strongly contracted, and alterations may be detected in other structures controlled by the autonomic nervous system.

Changes in the urinary bladder have been carefully studied. There is an immediate post-operative hyperactivity of the bladder muscle to stretch and peripheral stimuli. As a result, the vesical capacity decreases sharply and rhythmic waves of contraction of the detrusor appear. After several days the waves lessen in degree and the bladder slowly enlarges.

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FACTORS DETERMINING THE TYPE OF RESPONSE IN THE FIBER OF STRIATED MUSCLE

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The principle of gradation of action in skeletal muscle by the compounding of totally responding units was enunciated by Gotch (1902) and first demonstrated by Keith Lucas (1905, 1909). During the decade following the first confirmatory evidence for this all-or-none behavior of the individual fiber (Pratt, 1917) there was no similarly direct evidence to the contrary. Soon after the introduction, however, of the frog's retrolingual membrane as an object of study by Fischl and Kahn (1928), reports of graded activity came from various observers who used microelectrodes to stimulate the individual muscle fibers of this preparation: the type of response was of a peculiar localized character, which appeared to differentiate itself from the totally conducted all-or-none effect in that the graded contractions were confined to the region of the stimulating electrodes.

Two sets of conditions have been invoked as determining the occurrence of such local graded responses; those associated with the size and the mode of application of the electrodes (Gelfan, 1930; Gelfan and Gerard, 1930), and those arising from the state of the tissue (Hintner, 1930). When we consider, further, the contention that the total fiber response may also be capable of gradation (Brown and Sichel, 1930, 1936) it is not surprising that no consistent view of the character of these contractions has as yet been formulated. To accept the localized response as a normally occurring contractile mechanism is hazardous; yet to dismiss it as wholly abnormal (Kato, 1934) seems equally so. It is probably safest to regard it as a part-phenomenon, the study of which may aid in the analysis of the general process of excitation and contraction (Pratt, 1930).

In view of the present status of the subject, an attempt has been made to ascertain more exactly the conditions which influence the character of contraction and so determine the occurrence of the local response.

METHOD. All experiments were made on the single fibers in the retrolingual membrane of *Rana pipiens*. The method of preparation was essentially that described by Pratt and Reid (1930). A superficial median longitudinal incision was made in the "ventral" surface of the everted

tongue of the pithed frog. Under the dissecting microscope the tissue on both sides of the incision was carefully cut so as not to injure the large vessels of the rich bilateral blood supply. During this procedure the preparation was kept moist with Ringer. After a small opening had been made into the sinus basihyoideus, it was filled with a few drops of Ringer and the genioglossus and hyoglossus muscles were then cut transversely so that a larger field of the membrane could be obtained.

The frog was placed (prone position) in a Petri dish containing enough Ringer to cover the membrane. A block of thick glass was inserted from below into the opening in the sinus basihyoideus, so that the membrane rested upon its surface. The dish was set on the microscope stand and transilluminated from below.

Quartz covered platinum micro-electrodes (Taylor, 1925) were used to stimulate the fibers. Unipolar stimulation was first applied. By means of a micromanipulator the active electrode (0.25–4.00 μ diameter) was placed in position to stimulate a muscle fiber directly. Ordinarily a large indifferent electrode was placed in the bath covering the preparation. All the experiments were repeated with two stigmatic electrodes.

Break induction shocks were used at first. When local contractions were obtainable it was found that they could be produced both with cathodal and with anodal stimulation; but with anodal stimulation a stronger stimulus was necessary to elicit a response. Later, to control more readily the strength of the stimulus, condenser discharges were used. The two forms of stimulation gave similar results, and the experiments to be described were all carried out with the condenser-discharge stimulator (Schmitt and Schmitt, 1932).

RESULTS. *General factors. Normal preparation.* The threshold of the single muscle fiber of a normal fresh preparation with intact circulation is at first relatively high. After a short time the threshold drops considerably, and a weak stimulus is now sufficient to elicit the maximal response.

Gelfan (1930) and Gelfan and Gerard (1930) state that for the production of local graded contractions in a single fiber it must be stimulated with a micro-electrode in actual contact, and one preferably within a diameter of 2 to 5 μ (Gelfan, 1931). It appeared desirable to stimulate a fresh fiber, fulfilling these conditions, in order if possible to confirm the results.

With a fine micro-electrode (1 to 3 μ diameter) touching the fiber, a local contraction was promptly obtained in response to threshold stimulation. Invariably, however, there soon appeared a little wheal on the fiber in the region of the electrode, indicating that the fiber was injured. If the electrode was placed so as not to touch the quiescent fiber, yet close enough to touch when the fiber contracted, a wheal again appeared after

a short period of stimulation. A fiber stimulated in this way would again give graded contractions; but if the electrode was applied as close to the fiber as possible without touching when it contracted, no local responses were obtained to threshold stimulation. In one series of experiments, the fibers of fresh normal preparations with intact circulation were stimulated with a micro-electrode of 0.25 μ diameter applied in the manner just described. A sufficient interval (6 seconds) was allowed between stimuli to eliminate fatigue. The response to threshold stimulation or to repeated subminimal stimuli was always all-or-none. *Even after two hours of continued stimulation the contractions were persistently maximal.*

Effect of injury. Local graded contractions are readily produced in a fresh normal fiber that has been injured either by cutting or by piercing it with the electrode. The response of the fresh intact fiber is always all-or-none; but upon such injury the local graded contractions are obtainable. Hintner (1930) and Kato (1934) also observed these contractions in injured fibers. The local responses are usually preceded by contractures induced at the time of injury. Frequently, under these conditions, there appears a type of gradation which seems to involve the entire fiber: with a threshold stimulus there results an almost imperceptible movement of the whole, and, as the strength of the stimulus is increased, the contraction increases to a maximum. The increase in the strength of the contraction can be observed by noting the amount of displacement of a bent fiber with relation to a capillary that lies nearby. It is this form of gradation, presumably, that Brown and Sichel (1930) and Asmussen (1931, 1932) have recorded. Brown and Sichel (1936) obtained records of graded responses of single isolated fibers stimulated with electrodes placed on either side along the entire length of the fiber. From these records they conclude that the fiber as a whole is capable of gradation. I have observed, however, that this type of response disappears upon continued stimulation, and that local graded contractions are then obtained under weak stimuli.

The following representative experiment indicates the difference in response between normal fibers and fresh fibers that have been injured. A fresh preparation with intact circulation was made, and one side of the membrane was slit longitudinally. The cut fibers all gave local responses, while those that were intact gave only all-or-none contractions. The electrode was then placed so that it touched an intact fiber. Shortly afterward this fiber, which had been responding hitherto in an all-or-none fashion, now gave local graded responses. The characteristic wheal soon developed at the point where the electrode touched the fiber—the familiar sign of injury.

If fibers are stimulated too near a region of injury they will soon cease responding. But if the electrode is placed a little distance away from the

injured area, the fiber again responds with local contractions. Injured fibers soon become non-irritable and do not respond to further stimulation. Nevertheless, if they are allowed to recover, such fibers are again capable of responding. This response is at first only of an all-or-none character, but local contractions soon appear. The time necessary to produce the local responses depends on how long a non-irritable injured fiber is allowed to recover. If the fiber is left alone from ten to fifteen minutes it will respond with a few maximal contractions and then immediately give local responses. But if the fiber is allowed to stand from one to two hours before it is stimulated, only all-or-none responses are obtainable. It is only after continued stimulation for a few minutes that local contractions again appear.

Effect of contracture responses. Gelfan (1930) obtained local contractions by stimulating the fiber with a tetanizing current of short duration. With this type of stimulation I was likewise able to obtain local responses in a fresh fiber; but the fiber invariably responded with contractures before the local contractions appeared. If the tetanizing stimulus does not produce contractures, the fiber continues to respond only in an all-or-none manner: then, when contracture responses have been produced, local contractions appear upon repetition of the stimulus. A fiber that is giving nothing but maximal contractions can always be made to give local responses if it is stimulated with single shocks of sufficient strength to produce a contracture. The electrode may be placed so far away that it does not touch the fiber at any time, a precaution which eliminates any possibility of mechanical injury. If a fiber that has been thus conditioned by contractures to give local responses is allowed to remain briefly quiescent, it will revert to the all-or-none type of response. Nevertheless, such fibers will again give local contractions if stimulated for a short time.

Effect of fatigue. A fresh normal fiber that is giving only all-or-none contractions can be conditioned by fatigue to give local graded responses. A fiber was stimulated rapidly; with onset of fatigue, local contractions appeared under threshold stimulation. If this fiber was allowed to rest for a short time (about 15 minutes) before further stimulation, it would revert to the all-or-none type of response provided a sufficient interval elapsed between stimuli. But if the stimuli were sent in too rapidly, graded contractions were again observed. Although a fatigued fiber responds maximally after a period of rest, graded contractions will appear more readily in such a fiber than in a non-fatigued fiber under the same conditions of stimulation.

Frequency of stimulation. Since fatigue evidently can be one factor in the production of local responses, it remained to ascertain whether there is a critical frequency of stimulation below which only all-or-none responses are obtained, and above which local contractions appear. The

frequency of stimulation was controlled with a metronome having a mercury contact key. A micro-electrode of 2.5 μ diameter was used for these experiments. The time at which the preparation was made was recorded. The interval that elapsed between the making of the preparation and the time at which a given fiber was stimulated was also recorded, and is referred to as the "age of preparation" (table 1). The duration of

TABLE 1

EXPERIMENT	AGE OF PREPARATION	FREQUENCY OF STIMULATION (PER MINUTE)	DURATION OF CONTINUOUS STIMULATION BEFORE APPEARANCE OF LOCAL CONTRACTIONS
	minutes		
1	15	20	(At end of 90 min. no local contrac.)
1	106	20	(At end of 120 min. no local contrac.)
2	20	30	(At end of 103 min. no local contrac.)
2	124	30	(At end of 55 min. no local contrac.)
3	17	30	(At end of 123 min. no local contrac.)
4	14	33	15 min. 23 sec.
5	25	33	11 min. 37 sec.
4	30	33	12 min. 18 sec.
4	47	33	8 min. 9 sec.
5	53	33	7 min. 15 sec.
4	59	33	7 min. 53 sec.
4	77	33	3 min. 51 sec.
5	80	33	2 min. 41 sec.
4	83	33	3 min. 14 sec.
4	91	33	1 min. 57 sec.
6	9	35	10 min. 55 sec.
6	20	35	6 min. 13 sec.
6	27	35	4 min. 39 sec.
6	32	35	3 min. 8 sec.
6	36	35	1 min. 28 sec.
7	12	40	6 min. 47 sec.
7	20	40	2 min. 58 sec.
7	24	40	1 min. 12 sec.

stimulation was recorded with a stop-watch. The responses were constantly observed during the period of stimulation and the threshold frequently explored in order to maintain the liminal stimulus.

The data presented in table 1 have been compiled from seven different experiments and are typical of all the results in this series. It will be seen that a frequency of stimulation of 30 per minute will not produce local contractions in fresh uninjured fibers, whereas a frequency of 33

per minute will do so. The duration of stimulation necessary for the appearance of local contractions decreases as the frequency is increased above the minimum of 33 per minute. It will also be seen that with a frequency of 33 per minute the duration of stimulation shortens with the increasing age of the preparation. Another condition favorable to the production of local responses is, then, the advancing age of the preparation irrespective of the frequency of stimulation.

Time factor. Although it was found that fresh uninjured, non-fatigued fibers with intact circulation gave only all-or-none responses even with the smallest stimulating electrode (0.25 μ diameter), it was observed that the fibers of such a preparation a few hours old often gave local contractions. Usually the preparation was two or three hours old before local responses were obtained. This is confirmatory of the results of Hintner (1930) and of Kato (1934), who also found that these responses could be seen only in the older of uninjured preparations.

Another factor which must be taken into account is the presence of an intact circulation. Local responses were usually obtained much sooner in fibers which had no circulation. On the other hand, the preparation was in many cases five hours old before submaximal responses were observed in fibers which had an active circulation throughout this period.

The critical frequency of 30 per minute will elicit only maximal responses in fresh uninjured fibers. But after a few hours the conditions are favorable for submaximal responses irrespective of the frequency of stimulation, the critical frequency being no longer in evidence. In such a preparation the fibers will give local contractions when stimulated with a frequency of 20 stimuli per minute or even less.

Size of electrode. If a muscle fiber is capable of responding submaximally, the local contractions can be elicited even when the fiber is stimulated with a coarse electrode. Asmussen (1932) also was able to obtain graded responses in an isolated fiber with coarse electrodes; but Gelfan (1933) believes that these results were due to injury of the fiber during the process of dissection. Care was taken, therefore, not to injure any of the fibers that were being stimulated. This was accomplished by making an uninjured preparation and leaving it unstimulated for a few hours. Fibers of such a preparation readily gave graded responses when stimulated with a fine micro-electrode. A coarse electrode (100 μ diameter) was then substituted, and was applied as close to the fiber as was feasible without injuring it. Local responses were obtained by repetitive subminimal stimuli.

Gelfan and Gerard (1930) suggest that the local contractions are a result of direct stimulation of the contractile units with micro-electrodes, but that the conductile mechanism necessary to produce a maximal response is not activated when such a small area is stimulated. They

found that if the electrode was moved away slightly from the fiber the local contraction was eliminated and that the same stimulus then produced a maximal response. They state that since this type of stimulation is the more diffuse, a larger area is stimulated and there arises a propagated disturbance resulting in an all-or-none contraction. Microstimulation, with the electrode touching the fiber, is held by them to be necessary in order to obtain submaximal responses in both curarized and non-curarized preparations.

These results are not confirmed: I have observed graded responses when an effective stimulus was applied, regardless of the distance of the electrode from the fiber, provided the fiber was in a condition favorable for local contractions. When the micro-electrode was placed too far away from the fiber there was no response; but upon increasing the strength of the stimulus a submaximal contraction could be again elicited.

The above experiment was repeated with a coarse electrode (200 μ diameter). Uniform supramaximal stimuli were applied, and the dis-

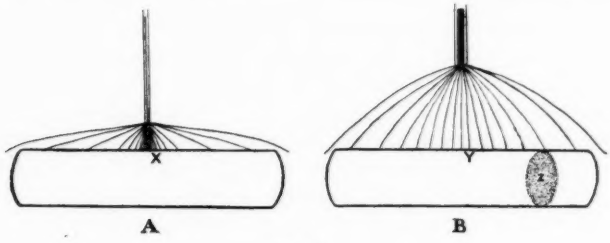

Fig. 1

tance between the electrode and the fiber was varied. When the electrode was near the fiber there was a maximal response. As the electrode was moved farther away, local responses were observed. As the distance was further increased, diminishing gradation of the submaximal response was obtained. Finally a point was reached beyond which there was no response. When now the distance between the electrode and the fiber was decreased, the response increased until a maximal contraction resulted. The same magnitude of response was obtained at any given point when the electrode was being moved either away from or towards the fiber.

The assumed distribution of current density is represented schematically in figure 1. It is seen that the farther away the electrode is moved, the greater is the spread of the stimulating current. But no matter how great the spread, the response is still submaximal in a fiber capable of giving such a response. (The fact that a local response is obtained at all is in direct opposition to the view of Gelfan and Gerard, who claim that any

amount of current spread must under such conditions produce a maximal response.) And if a fiber is responding in an all-or-none manner the response is independent of the size of the electrode or the distance between the electrode and the fiber. An effective stimulus always produces a maximal response.

The greatest current density is directly beneath the electrode. There is a greater current density in A (fig. 1) than in B, since in the former case the electrode is nearer the fiber and there is not so great a current spread. Also at a given point x (A, fig. 1) the current density is greater than at the corresponding point y (B, fig. 1). One might offer the objection that although a large area is being stimulated when the electrode is at a distance from the fiber, the intensity of the stimulus at any point is not great enough to evoke a propagated disturbance. If this were the case, all the local responses to an effective stimulus should occur directly beneath the electrode since the current density is greatest in this region of the fiber. Nevertheless, submaximal responses were observed a short distance away (z, fig. 1, B) where the current density is undoubtedly not so great as it is directly beneath the electrode. This type of response will be described in detail in a later paper.

The results of these experiments indicate that the local response is dependent neither upon the size of the electrode nor upon its distance from the fiber. If a fiber is capable of responding locally, the local graded contractions can also be obtained with a coarse electrode applied at some distance from the fiber.

Effect of constant strength of stimuli. Gelfan and Gerard (1930) have pointed out that the magnitude of the local response is dependent on the strength of the stimulus. If a fiber is stimulated under the conditions they specify, namely with a micro-electrode touching the fiber, a weak stimulus will elicit a local contraction which is constant for the given stimulus.

The conditions stipulated above were rigidly adhered to, and micro-electrodes of 0.25 to 2.00 μ diameter were used. Once the micro-electrode was placed in position, it was not disturbed throughout the experiment. The muscle fiber was stimulated with condenser discharges, the strength of which could be controlled. By means of a cathode-ray oscillograph it was determined that for any given adjustment of strength the condenser discharge to be used as a stimulus remained constant. The frequency of stimulation was below the critical frequency of 33 per minute. A fresh uninjured fiber always responded maximally; but when the fiber was capable of responding locally, the response at first varied although the strength of the stimulus was uniform. With a strength of stimulus sufficient to elicit local contractions, the response to the first stimulus might be local while the second and third stimuli might evoke

maximal responses; the fourth stimulus might again give a submaximal contraction, etc. Upon continued stimulation, however, the maximal responses became fewer. Soon they disappeared, and the same stimulus now elicited only local contractions. With further stimulation these also disappeared and the fiber did not respond at all. It appears, therefore, that the contractile elements responsible for the local contraction can be fatigued. If the stimulus was now slightly increased, only maximal responses were obtained. After a short period of rest, local contractions again appeared. Moreover, if the strength of the stimulus that was producing only local contractions was kept constant, but its duration increased, the fiber would give maximal responses.

Control through balance of inorganic salts. The recognized importance of potassium in the excitation process suggested the desirability of studying the effect of excess potassium on single muscle fibers. Experiments undertaken to this end yielded the following results.

The fibers of a fresh preparation were found as usual to respond with the all-or-none effect. The normal Ringer bathing the preparation (made up as in previous experiments: 0.65 per cent NaCl; 0.014 per cent KCl; 0.012 per cent $CaCl_2$; 0.003 per cent $NaHCO_3$) was then changed for one containing an excess of KCl. It was found that a solution containing an excess of 0.028 gram KCl per 100 cc. normal Ringer readily produced local contractions in fresh fibers (which were responding maximally in normal Ringer). If the preparation was now placed in normal Ringer for a time, the fibers again responded maximally; but after a short delay, submaximal responses were again obtainable. At the same time that the earlier of these results were announced (Steiman, 1935), Zwikster and Boyd (1935) reported that the hearts of the turtle and frog respond in a graded fashion to strong graded galvanic currents when immersed in excess KCl-Ringer, the effect being reversible when the solution is changed for normal Ringer.

Since normal Ringer was temporarily effective in eliminating graded responses, it seemed probable that an excess of NaCl (since it is antagonistic to KCl) would entirely eliminate submaximal responses due to treatment with KCl. It was found that the graded contractions observed in fresh fibers which were immersed in the KCl-Ringer could be immediately eliminated by changing the solution for normal Ringer plus 0.33 gram NaCl per 100 cc. Under these conditions only maximal responses were obtained. If the preparation was now replaced in the KCl-Ringer, the local responses soon returned. In the NaCl-Ringer the fibers again responsed with maximal contractions. Thus the effect is reversible at will.

A series of experiments of the following type were made in order to study the responses of fibers of the same preparation, different fibers being

severally under different conditions while the effects of KCl and NaCl were determined.

A fresh preparation was made, and one side of the membrane was slit along its lateral attachment. The injured fibers gave local responses, while those that were intact responded maximally. The preparation was then placed in Ringer containing an excess of 0.028 gram KCl per 100 cc. The fibers on the intact side now likewise gave local responses. When the solution was changed to Ringer containing an excess of 0.33 gram NaCl per 100 cc. the intact fibers again responded maximally, but the injured fibers still gave local responses. The bath now being changed to normal Ringer, a few of the intact fibers were stimulated with supramaximal stimuli, resulting in contracture responses; and local contractions soon appeared. Other intact fibers were injured with the electrode and local responses were also obtained, while still other intact fibers were stimulated rapidly up to the onset of fatigue, with the appearance of local contractions. The preparation was now immersed in a bath containing an excess of NaCl; but the graded responses still persisted in all fibers which were subject to the treatment described above. The remaining intact fibers, which neither were fatigued nor had been made to give contracture responses, responded maximally. However, after the preparation had been standing for a few hours they too responded locally. The NaCl-Ringer was effective, nevertheless, in eliminating the local contractions occurring in the intact fibers (as a result of age); only maximal contractions were then obtained. Thus an excess of 0.33 gram NaCl per 100 cc. Ringer is effective in eliminating local contractions from muscle fibers of old preparations. But the submaximal responses characteristic of fatigued or injured fibers cannot be thus eliminated.

Since the anion is the same in both NaCl and KCl, the effects produced by these salts must be due to the Na and K ions. It was thought, however, that since osmotic conditions are changed by the addition of either salt to normal Ringer, the results might in part be due to a change in osmotic pressure. Consequently, the effect of Ringer with unaltered osmotic pressure and with an excess of either NaCl or KCl was determined. In the NaCl-Ringer the amount of KCl was proportionally reduced so that the total osmotic pressure remained the same. Likewise with the KCl-Ringer, the NaCl content was lowered. It was found that these solutions were equally as effective as those in which the osmotic conditions were altered. It must be concluded, then, that the production and elimination of local contractions in single muscle fibers can under certain conditions be controlled by changing the electrolyte composition (Na and K) of the bathing medium.

Effect of curare. Gelfan states that in curarized preparations micro-

stimulation again is necessary to obtain submaximal responses. He (1933) objects to Asmussen's view (1932) that the all-or-none property of the muscle fiber is a function of the end-plate, on the basis that if this were the case microstimulation would not be necessary to produce graded responses in curarized fibers, since curare eliminates the end-plate.

All the previous experiments were repeated with curarized preparations and the same results were obtained. Curarization was held to be complete when stimulation of the nerve failed to give a response of the muscle fiber. It was found, however, that the fibers of such curarized preparations, which were under conditions in which they responded locally to microstimulation, also responded locally when stimulated with coarse electrodes. The fiber was always stimulated at a distance from the point of entrance of its nerve.

If the end-plate be a factor eliminated by curare, and assuming Asmussen's hypothesis to be valid, then graded responses alone should result with direct stimulation. It was found, however, that a uniform strength of stimulus applied at a point on the fiber distant from its area of innervation could produce indifferently either local or maximal contractions. The objection might be advanced that curare does not completely eliminate the end-plate factor assumed responsible for the all-or-none property of the muscle fiber. If this were so one should expect, then, only maximal contractions, in accordance with Asmussen's hypothesis. Nevertheless, if the conditions are favorable for local contractions, even under incomplete curarization, the fiber will respond locally. And if the conditions are not favorable for local contractions, only maximal responses are obtained with threshold stimulation in fibers of either curarized or non-curarized preparations, irrespective of the size of the electrode.

Discussion. The response of a fresh normal, intact fiber is always maximal. Nevertheless, this all-or-none behavior is modified through the operation of a variety of factors. Some of these are contracture, fatigue, age of preparation, lack of circulation, and injury.

The findings here reported indicate that the occurrence of local graded contractions is not limited by the size of the electrode or by the strength of the stimulus. Gelfan and Gerard (1930) suggest that the local responses are due to a localized stimulus directly exciting the contractile units without arousing the propagated disturbance, and state that if the electrode is slightly removed from the fiber, the same stimulus now causes a maximal response since a larger area of the fiber is stimulated. They conclude that the propagated disturbance is concerned with diffuse stimulation which produces only maximal responses. Since in the present work with the normal intact preparation no local responses have been obtained, the criticism might be advanced that the fiber was stimulated without contact with the micro-electrode. Yet it is found that if the condition of the fiber

is such that local contractions are obtainable, they can be elicited with coarse electrodes and diffuse stimulation. Furthermore, if the size of the local response were solely a function of the strength of stimulus, a stimulus small enough to produce a local response should at any given time elicit local contractions only. However, it has been shown that during the period when local contractions first appear, the *same* strength of stimulus will elicit either a local or a maximal contraction, depending apparently on the condition of the fiber at the moment.

That the production and elimination of local contractions can under definite conditions actually be controlled by changing the electrolyte composition (Na and K) of the bathing medium, is further evidence that this response is not entirely determined by the character of stimulation.

Although the local response may be due to direct stimulation of the contractile units, it can be produced with either a localized or a diffuse stimulus. It would seem that the local contraction is again not dependent on the manner of stimulation but appears when a separation has taken place between the conductive mechanism for the propagated disturbance and the contractile elements. The local contraction is itself twitch-like, and has been shown by Gelfan (1933) to have the same form and duration as has the all-or-none contraction. It might well be that the local contraction is a miniature of the integrated maximal response of the whole fiber; that normally this response is masked, but that under conditions which derange the normal properties of the conducted disturbance—reflected in a disjunction of the integrated units—the local contraction appears as a separate entity.

SUMMARY

1. In a fresh uninjured preparation the responses of single muscle fibers of the frog's retrolingual membrane were always maximal, even when micro-electrodes as small as 0.25 to 4.00 μ diameter were used.

2. Absence of circulation, injury, incident contractures, and the age of the preparation are some of the conditions found to be favorable for the production of local graded contractions.

3. A fresh fiber which is fatigued will give local responses. A frequency of 30 stimuli per minute gave maximal responses even after two hours of continuous stimulation; while a frequency of 33 stimuli per minute soon caused local contractions. The time necessary for their appearance shortened with the age of the preparation and with the increase in frequency of stimulation above the minimum of 33 per minute.

4. A fiber responding locally to a given strength of stimulus may also respond in an all-or-none manner to the same stimulus.

5. A coarse electrode is capable of eliciting local contractions in the

single fiber. With an electrode of 200 μ diameter, the magnitude of the local response diminished as the distance between the electrode and the muscle fiber was increased.

6. The fibers of a fresh preparation which had been responding in an all-or-none manner gave local contractions when the Ringer was modified by addition of 0.028 gram KCl per 100 cc. Such contractions, as well as those obtained from fibers of an old preparation, were immediately eliminated (with reversion to the all-or-none type) by changing the solution for Ringer plus 0.33 gram NaCl per 100 cc. This effect was reversible. The above change did not, however, eliminate the local contractions obtained from a fatigued or injured fiber.

7. All the results were obtained equally well with fibers of curarized preparations.

I wish to acknowledge my indebtedness to Dr. F. H. Pratt for his advice and criticism.

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THE INFLUENCE OF PYLORECTOMY UPON THE STRENGTH OF THE ACID SECRETED BY THE FUNDUS

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In previous publications (1, 2) it was shown that the *total amount* of acid secreted by whole stomach pouches from which the pylorus had been removed was only about $\frac{1}{5}$ to $\frac{1}{6}$ as great as by pouches in which the pylorus was in situ. Likewise in the intact animal it was shown that following partial gastrectomy (pylorectomy), the acidity of the total secretions entering the stomach and of the gastric contents was definitely lower than could be accounted for by the diluting and neutralizing effects of the duodenal secretions entering the stomach. In addition to these changes in acid secretion it was noted that in the intact animal following partial gastrectomy, the consistency of the gastric contents was greatly altered due to the presence of large amounts of mucus resembling egg albumin. The amount of this mucus was in a general way inversely related to acid secretion; when acid secretion was elevated by histamine or the intestinal phase, the mucus was absent or small in amount, while when the acid secretion dropped to low levels large amounts of mucus were present. This change in mucus content of the secretion was not noted in pylorectomized whole stomach pouches.

In the above studies no attempt was made to analyze the exact nature of the changes in acid secretion, that is, to determine whether there was simply a decrease in the total amount of acid secreted, the strength of the acid remaining normal, or whether there was actually a decrease in the strength of the acid secreted by the fundic glands. In the present paper we wish to present an analysis planned to elucidate the nature of the changes in acid secretion.

MATERIAL. *Whole stomach pouches.* A total of 97 half-hour gastric samples was used from nine whole stomach pouches. Forty-two samples were from four pouches in which the pylorus was in situ and 55 samples were from five pouches from which the pylorus had been removed. Three of the pouches in each group had the entire nerve supply intact, while the remainder were partially (vagus) denervated.

Intact dogs before and after partial gastrectomy. A total of 362 half-hour gastric samples was obtained from six dogs before and after removal of the pylorus and anastomosis of the fundus to the duodenum (Polya type). One hundred forty-five half-hour samples were obtained before operation and 217 samples after operation. In four of the dogs the vagus nerves were

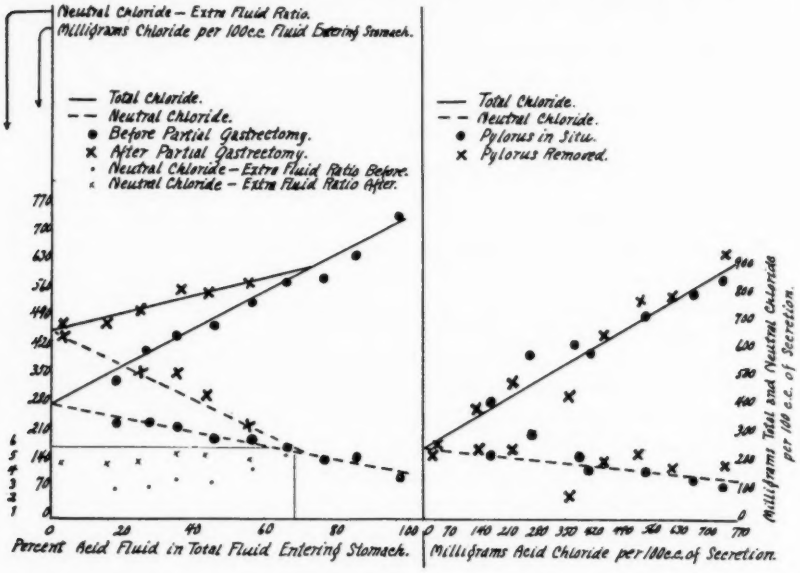
Neutral Chloride – Extra Fluid Ratio.
Milligrams Chloride per 100 c.c. Fluid Entering Stomach.
Total Chloride.
Neutral Chloride.
Before Partial Gastrectomy.
After Partial Gastrectomy.
Neutral Chloride – Extra Fluid Ratio Before.
Neutral Chloride – Extra Fluid Ratio After.
Total Chloride.
Neutral Chloride.
Pylorus in Situ.
Pylorus Removed.
Milligrams Total and Neutral Chloride per 100 c.c. of Secretion.
Percent Acid Fluid in Total Fluid Entering Stomach.
Milligrams Acid Chloride per 100 c.c. of Secretion.

Fig. 1. *Left half.* An analysis of 362 half-hour gastric samples obtained from 6 dogs before and after partial gastrectomy. The samples were grouped according to increasing percentages of acid fluid in the total fluid entering the stomach. The average total and neutral chloride concentrations for each group were plotted against the corresponding averages for the per cent of acid fluid. The neutral chloride-extra fluid ratios are shown in lighter ensignia in the lower left hand corner.

Right half. An analysis of 97 half-hour samples obtained from 4 whole stomach pouches with the pylorus in situ and 5 with the pylorus removed. The samples were grouped according to increasing acid chloride concentrations in the secretion and the average total and neutral chloride concentrations for each group plotted against the corresponding average acid chloride concentration.

cut in the thorax before partial gastrectomy while in two others the vagi were intact.

METHODS AND RESULTS. All studies were made with a two per cent Liebig's extract test meal containing 15 mgm. of phenol red per liter (3, 4). The fractional method of analysis was used in most tests.

A. *Whole stomach pouches.* The samples obtained from the pouches with the pylorus in situ were grouped according to increasing concentra-

tions of acid chloride per 100 cc. of secretion entering the pouch. The average acid chloride, neutral chloride and total chloride per 100 cc. of secretion were determined for each group. Similar group averages, using the same increment for acid chloride, were prepared for the samples obtained from the pylorectomized pouches. The group averages for the total and neutral chloride concentration were then plotted against the corresponding average acid chloride concentrations. The result is shown in the right half of figure 1 where it is seen that for the same acid chloride concentration of the secretion, the total and neutral chloride concentrations are the same in pouches with and without the pylorus. This agreement indicates that the acid chloride concentration of the pure acid secretion was the same in both types of pouches. If the acid chloride concentration of the pure acid secretion had been definitely lowered by pylorectomy, then samples containing the same acid chloride concentration as samples from non-pylorectomized pouches would show lower total and neutral chloride concentrations. It thus appears that the marked decrease in the gastric acidity curve which occurs after pylorectomy (1, 2) is due simply to a decrease in the *amount* of acid secretion, the *concentration* of the acid secretion remaining unaltered.

In a previous publication (3) it was shown that when a two per cent Liebig's extract test meal is used in whole stomach pouches, water is absorbed from the pouch contents during the course of the experiment. This accounts for the higher ranges of the chloride fractions seen in figure 1. Fourteen samples had values for the acid chloride concentration in excess of 751 mgm. per 100 cc. (11 samples from pylorectomized pouches and 3 samples from normal pouches). These samples were not used in the present analysis. The evidence of water absorption seemed to be about the same whether the pylorus had been removed or was in situ, hence whatever changes may be attributed to this factor, probably influence both sets of data to about the same degree.

B. *Intact animals before and after partial gastrectomy.* The samples obtained before partial gastrectomy were grouped according to increasing percentages of acid fluid in the total fluid secretions entering the stomach. (This is virtually the same as grouping according to increasing concentrations of acid chloride in the secretion as was done with the samples from the whole stomach pouches.) The average values for the per cent of acid fluid and the milligrams of total and neutral chloride per 100 cc. of secretion were calculated for each group. Similar group averages were prepared from the samples obtained after partial gastrectomy. The average concentrations of total and neutral chloride were then plotted against the corresponding averages for the per cent of acid fluid. The result is shown in the left half of figure 1. It is seen that for the same percentage of acid fluid in the secretions entering the stomach, the total and neutral chloride

concentrations are definitely higher after partial gastrectomy than before. For zero acid fluid the total and neutral chloride values unite at 280 mgm. per 100 cc. before and at 460 mgm. after operation. For the higher ranges of acidity the total and neutral chloride concentrations appear to be the same before and after operation.

The interpretation of the above findings is somewhat difficult. However, if we grant that the concentration of the pure acid secretion is unaltered by pylorectomy, as indicated by the analysis of the whole stomach pouch experiments, then the changes noted here would be due entirely to a higher neutral chloride concentration of the non-acid fluid entering the stomach after partial gastrectomy. This conclusion is supported by the fact that the neutral chloride-extra fluid ratios (lower left hand corner) are considerably higher after operation than before. The increase in the neutral chloride concentration of the non-acid fluid is undoubtedly related to and dependent upon the thick tenacious mucus which appears in the gastric secretions after partial gastrectomy and which is most marked when the acid secretion is low but tends to disappear when the acid secretion is high.

DISCUSSION. We believe that the analysis presented above justifies the following conclusions: After removal of the pylorus the concentration of the acid fluid secreted by the fundic glands remains unchanged. However, due to the removal of the specific stimulating influence of the pylorus, the *quantity* of the acid fluid secreted is definitely reduced. In whole stomach pouches, the mucus secretion is not altered, at least not during the period of observation used in these studies. Following partial gastrectomy, in the dog, there is in addition to a decrease in the amount of acid secretion, a change in the character of the mucous secretion, so that it becomes very tenacious and thick and its neutral chloride concentration increases. The apparent amount of this thick mucous secretion is inversely related to the amount of acid secreted, being most abundant when the acid secretion is low.

The findings in relation to the acid secretion reported in the present paper apparently explain a confusing finding noted by other workers. In the excellent paper by Steinberg, Brougher and Vidgoff (5), the acid secretion of a Pavlov pouch, in response to a meat meal in the main stomach, was studied before and after pylorectomy with a Polya anatomosis. They found that the *acidity* of the secretion from the pouch was unchanged by pyloric removal but that the *quantity* of the secretion was reduced to less than one-half the amount obtained before pyloric removal. This is in harmony with the findings outlined above.

The changes in mucous content are difficult to explain. In vitro experiments showed the mucus to be soluble in tenth normal hydrochloric acid. Since the presence of the mucus varied inversely with the acidity of the

gastric contents, it appears quite likely that the changes are dependent upon the solubility or non-solubility of the mucus in the gastric contents rather than to a true increase in mucous secretion when the acidity of the contents is low. This interpretation is in agreement with the statement of Bloomfield and Polland (6) who say that while the mucus is relatively increased in gastric anacidity, they have practically never observed any absolute overproduction of it under the conditions of the histamine test. The mucoid character of the secretion has, however, been repeatedly noted by many observers.

SUMMARY

An analysis of experiments performed on whole stomach pouches with and without pyloric removal and on dogs before and after partial gastrectomy shows that removal of the pylorus produces the following changes in gastric secretion:

1. A decrease in the *quantity* but no change in the *concentration* of the acid secreted by the fundus.

2. Following partial gastrectomy the gastric contents contain large amounts of a thick viscid mucus when the acid secretion is low but the mucus tends to disappear when the acid secretion is raised by histamine or the intestinal phase. This mucus is rich in neutral chloride and when present the total and neutral chloride concentration of the secretion is higher than normal. This mucus was not noted in whole stomach pouches from which the pylorus had been removed.

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LOCAL STIMULATORY INACTIVATION WITHIN THE CEREBRAL CORTEX, THE FACTOR FOR EXTINCTION[1]

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Variation of response is a common observation in experiments with electrical stimulation of the motor cortex. In 1881 Bubnoff and Heidenhain (3) discovered facilitation and inhibition. The following year Exner (8) gave additional evidence. Graham Brown and Sherrington (11) made a special study of the instability of the motor responses elicited on stimulation of one focus through simultaneous or antecedent stimulation of a second allied or antagonistic focus. One of us with Marshall (4) and with Brody (1) gave additional evidence of the modifiability of the responses elicited on stimulation of one motor focus under various experimental conditions.

However, if all experimental conditions, both of cortex and stimulation, are kept constant and the intervals between successive stimulations are long enough, the responses obtained upon stimulation of one motor focus are remarkably steady, except for the periodic "wave"-like fluctuations in the size of the responses, which Brody and one of us (1) have described.

While studying facilitation, the present writers came upon a new phenomenon, which they have designated *extinction*.[2]

The original observation was that an electrical stimulation of a motor focus, although effective when applied alone, was ineffective, when it followed 13 seconds after an identical stimulation.

The present paper deals with experiments showing that extinction originates in the cortex and is determined by physiological factors and by the characteristics of the electrical stimulation.

METHODS. The animals used were Macacus rhesus monkeys.

Anesthesia. The experiments were performed under anesthesia, usually "Dial" (Ciba),[3] supplemented if necessary with ether. In a few experiments ether was used exclusively, for a special purpose. The most even anesthe-

[1] The expenses of this investigation were defrayed from a grant of the Fluid Research Fund of the Yale School of Medicine.

[2] A brief account of the fundamental findings has been published in Proc. Soc. Exper. Biol. and Med. **32:** 524, 1934. See also This Journal **113:** 97, 1935, and Resumé of Abstracts, XV. International Physiol. Congress, Leningrad, Moscow, **1935**, 81.

[3] We wish to tender our thanks to the Ciba Co., New York, who kindly put the Dial at our disposal.

sia over many hours and the best excitability of the cortex are obtained if two-thirds of the dose of Dial (0.45 to 0.5 cc. per kgm. body weight) is given intraperitoneally and one-third intramuscularly. In this way the animals have been kept under even anesthesia as long as 36 to 58 hours, with a little additional Dial given intramuscularly on the second and third days.

If the whole dose of Dial is injected into the peritoneal cavity, it is often found that the animal, although fully anesthetized, is peculiarly jerky. The cortex then reacts very readily to electrical stimulation with after-discharge and the margin between liminal stimuli and those eliciting after-discharge is very narrow. These disturbances are nearly always obviated by administering the Dial as stated above.

Electrical stimulation.[4] In experiments of this kind, as in all stimulation experiments on the "finer" functional mechanisms of the cerebral cortex, it is absolutely necessary to have rigidly controlled stimulation. Although the existence of extinction can be demonstrated with manually applied unipolar or bipolar faradic stimulation, its investigation can only be undertaken with fixed electrodes and with exactly controlled, perfectly regular and equal "pulses." Therefore a thyratron stimulator, like that designed by Schmitt (15), was used. This device permits independent variation of the number of pulses per second (pattern-frequency) and the shape and duration of the individual pulses (pulse-frequency). Various kinds of biphasic, monophasic (half rectified) pulses with large and small, polarizable and non-polarizable electrodes have been tried in bipolar and unipolar stimulation, and all produce extinction. The usual procedure has been unipolar stimulation with thyratron pulses, a stigmatic silver–silver chloride electrode on the cortex, the large electrode in the rectum.

The durations of the periods of stimulation and the intervals between them were rigidly controlled by a mechanical device, two rotating drums with cams and jacks for contact, driven by a synchronous A.C. motor. The contacts on each revolving drum actuated a relay, throwing a mercoid switch and thus putting the stimulation through to the cortex. Later this device was abandoned for another one, designed by one of us (W. S. McC.), which gives a more flexible control of these time relations.

As this timing-device has proved to be very successful and reliable in its operation and has many applications in all kinds of stimulation experiments, we wish to describe it here briefly. Figure 1 gives a diagram of the apparatus and of the circuit used.

A revolving contact arm *1*, grounded through the apparatus, is driven through reduction-gears by a resistance-controlled, *2*, A.C.-motor, *3*, and touches successively

[4] The writers wish to express their thanks to Mr. Cecil T. Lane, Research Assistant in Biophysics of the Yale School of Medicine, for his valuable coöperation in all physical problems.

a number of insulated contact points (A_1, A_2, B_1, B_2, C_1, C_2). All but the last two of these are mounted on a brass disc, *4*, graduated in degrees and coaxial with the contact arm. The last two contacts are mounted on a segment, *5*, below the disc; of these the first, C_1, is fixed to the end of the segment and the second, C_2, is mounted on a clamp movable on the segment. Contact B_2 is fixed on the disc at the zero point of the graduation. The other three contacts (B_1, A_1, A_2) are mounted on clamps movable on the disc. The disc can be rotated manually into any given position and fixed there by a screw at *6*.

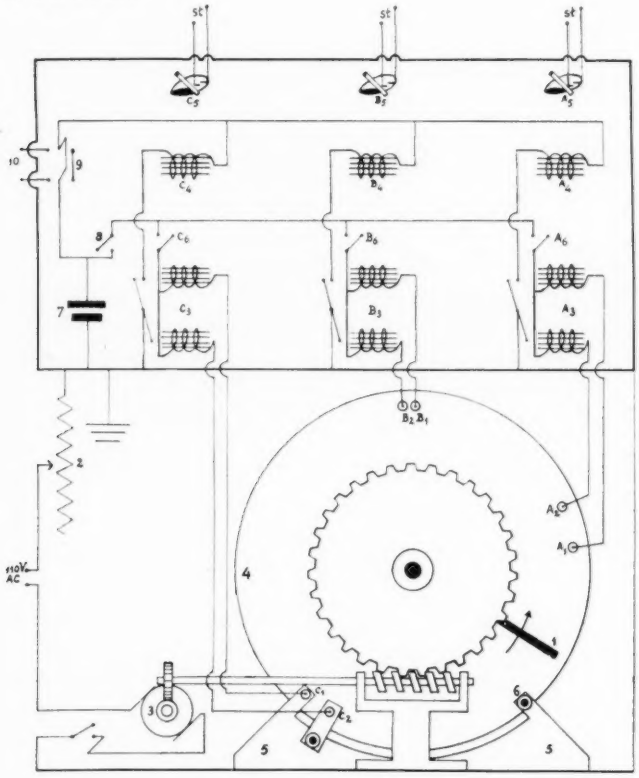

Fig. 1. Diagram of apparatus and circuit used. Description in text

By the use of the lock-in relays A_3, B_3 and C_3, part of the current from a 6-V storage battery, *7*, is allowed to flow through the electromagnets (A_4, B_4, C_4) controlling the mercoid switches A_5, B_5 and C_5 through which passes the stimulating current, *st*. In this way the stimulation lasts from the moment the contact-arm touches the first until it touches the second contact point of any given pair. Additional switches, A_6, B_6, C_6, are incorporated so that each stimulation or all, *8*, may be omitted. A double pole-double throw switch, *9*, is included so that the set may be turned off or operated with or without a signal marker (attached at *10*) in series with the electromagnets A_4, B_4, C_4. The whole relay-system is shielded and grounded.

In this way two or three stimulations are possible, in which the duration of each stimulation and the intervals between them can be varied independently; the duration of each stimulation is determined by the separation of a given pair of contact points, the interval by the distance between pairs.

The pulses from the source of stimulation were led to the input-terminals of a 10000-ohm precision decade voltage-divider and from its output-terminals to the leads, *st*, of the mercoid switches of the timing device. The voltages of the various stimulations are given in terms of the readings (in ohms) on the voltage-divider.

The responses elicited by the electrical stimulation of the "motor" cortex were recorded isotonically by tambours on smoked paper together with the periods of stimulation and a "time-line."

The pulse- and pattern-frequencies of the stimulations were repeatedly checked with the cathode ray oscillograph. In many instances the resistance and capacity of the animal were measured during stimulation (12), thus making it possible to determine the characteristics of the actual electrical stimulus to the organism.

RESULTS. Under the rigidly controlled conditions of these experiments the responses obtained upon electrical repetitive[5] stimulation of a focus of

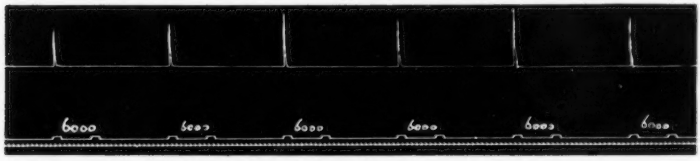

Fig. 2. Six pairs of identical stimulations (thyratron-discharges, 53 per sec.). Interval between the mates of each pair, 13 seconds. Intervals between pairs, about 28 to 30 seconds. First response of each pair large, second response absent (total extinction). Time at bottom (2 sec.).

the "motor" cortex, especially of area 4 (Brodmann), are remarkably uniform over many hours, except for the periodic fluctuations or "waves" in the size of the responses. The only other condition which must be fulfilled is that the intervals taken between the individual stimulations must be large enough. The depth of narcosis is the chief factor which determines the length of this interval, although other factors, namely, the characteristics of the stimulation, are also of importance. Under moderate depth of narcosis an interval of one minute is usually found to be satisfactory.

If the interval between two equal successive stimulations of a motor focus of an animal under average depth of narcosis is reduced to about 13 seconds, it is found that the second response is much smaller than the first or is entirely absent. Apparently an extinguishing influence from the

[5] We do not wish to speak here of faradic stimulation, reserving this term for the stimulation with the ordinary inductorium.

first stimulation is still active after that interval, resulting in a temporary absence or diminution of response to the second stimulation of that focus (see fig. 2).

In figure 2 the second response of each pair is extinguished (total extinction). That the first responses of each pair are large shows that the extinguishing factor active during each of the second responses has ceased to be effective after about 30 seconds, demonstrating how temporary is the activity of this extinguishing factor.

If extinction is partial, the size of the second response is not only diminished but its latency is markedly increased. This is shown in figure 3, which includes a pair of responses to equal stimulations at an interval of 13 seconds taken on a faster drum.

Fig. 3. Two pairs of equal stimulation (faradic stimulation, coil dist. = 10.9 cm.). Partial extinction of second response. Left pair on slow drum, second pair on faster drum to show increase in latency of extinguished response.

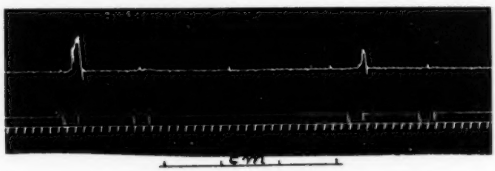

Fig. 4. Two pairs of identical stimulations, showing presence of extinction after laminar thermocoagulation (70°C. for 3.5 sec.) of outer three layers of cortex. Time line gives 2 seconds.

Extinction is a constant phenomenon, regularly observed in all experiments since it was first seen. It is present under Dial anesthesia, under mixed Dial-ether anesthesia, under ether alone and in the non-narcotized animal (see below). It has been observed with all forms of electrical stimulation mentioned in the paragraph on methods. Finally it can be stated that extinction has been observed and recorded for foci yielding extension, flexion or any other movement of any portion of either limb or of the face.

Extinction is a cortical phenomenon. It is still present, apparently unchanged, in a motor cortex of which the outer three layers have been killed by laminar thermocoagulation at 70°C. for three seconds (see fig. 4).

When, however, the whole thickness of the motor cortex has been killed by thermocoagulation (at 80°C. for 5 seconds) or when it has been removed

surgically, extinction is no longer demonstrable. In fact, repetition of the same stimulation to the fibers of the white matter underneath the cortex, at the same interval which on the normal cortex produced extinction, now gives rise to facilitation (see fig. 5).

These findings prove not only that extinction is a cortical phenomenon, but that it originates in the deeper layers of the cortex. Former stimulation experiments of one of us (5) and histological studies of the structure of the cortex implicate the layer of the large and giant pyramidal cells. It has been found that extinction is a very local phenomenon, a slight displacement of the electrode between stimulations being sufficient to miss it. This is the reason why manually applied stimulations produce less certain results.

Extinction is a phenomenon which is influenced by the physiological condition of the cortex and by the physical characteristics of the stimulation.

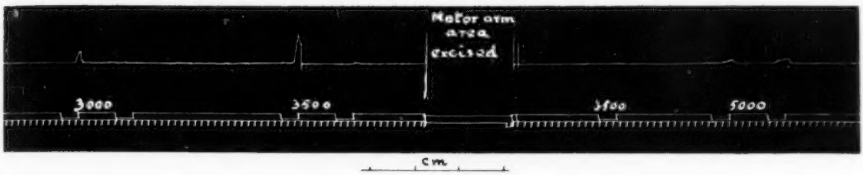

Fig. 5. In left half of this figure extinction from normal cortex. In right half extinction is superseded by facilitation (at same interval) on stimulation of fibers of corona radiata after extirpation of motor arm area.

I. *The condition of the cortex.* The functional condition of the cortex has a great influence on extinction. All conditions, such as deep anesthesia, poor circulation, cooling, which tend to depress or impair the functional activity of the cortex prolong the interval at which extinction is optimally demonstrable. The effect of a slight increase in the depth of narcosis is shown in figure 6.

The animal was under general Dial-anesthesia, which was fairly light, as is shown by the occurrence of a few spontaneous movements (sp). Figure 6 shows three pairs of stimulations, all of equal strength (5000), the intervals between the stimulations of each pair being the same (11.5 sec.). The first pair shows partial extinction of the second response, before, at the arrow in the figure, a pad of cotton wool with a little ether was held in front of the animal's nose. Thirty-four seconds later another pair of stimulations was administered. Now the second response is equal in size to the first. Then, after another 45 seconds, i.e., 91 seconds after the beginning of the etherization, repetition of a pair of stimulations results in a second response bigger than the first (facilitation). This means that under

deeper narcosis facilitation replaces extinction at this interval, and the optimal interval for extinction is increased. Under very deep narcosis extinction may be demonstrable with intervals up to 3 minutes.

Fig. 6. This figure demonstrates influence of slightly deeper anesthesia on extinction. Three pairs of identical stimulations with identical interval. In first pair extinction. In second pair equality of the two responses; in third pair facilitation.

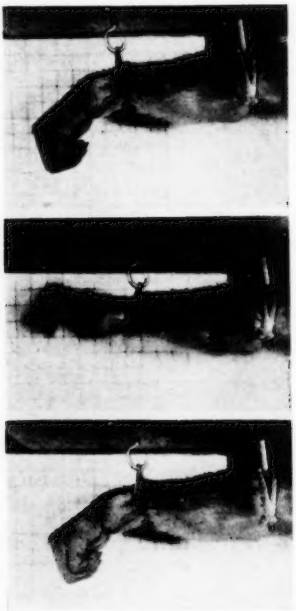

Fig. 7. Almost complete extinction in unanesthetized monkey. Upper photo shows resting position of hand. Middle photo shows maximum of response to first stimulation (at its end). Lower photo shows almost complete absence of response at end of second identical stimulation. Interval between these stimulations 6 seconds.

In the absence of narcosis a shorter optimal interval for extinction is to be expected. To verify this two experiments were made, in which the operation was performed while the animal was under ether narcosis, but stimulation withheld until he was awake. In these experiments the monkey sat in a box with one of his arms fastened so that it could be photo-

graphed or its movements recorded. To ensure the fixity of the electrode on the cortex, its holder was screwed into the skull. Figure 7 demonstrates the presence of extinction in the waking animal. The upper photo shows the resting position of the arm. The following photos give the dis-

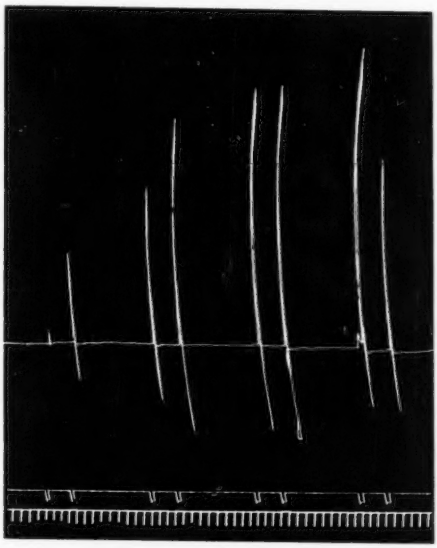

Fig. 8. Four pairs of identical stimulations, except for duration, showing facilitation with shorter, extinction with longer duration. Voltage in all 4 pairs 5450. Time-line marks 5 seconds.

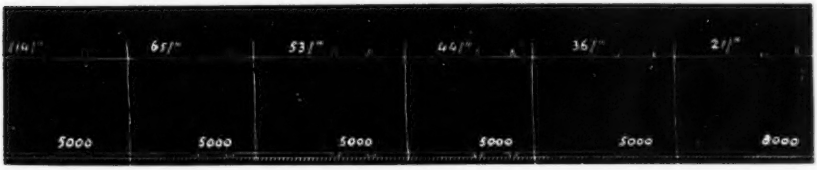

Fig. 9. This figure shows transition from extinction to facilitation with decrease of pattern-frequency. In the first 5 records the voltage used was the same, in the 6th record the voltage had to be increased to 8000 to obtain a response to the first stimulation of that pair.

placement of the hand at the height of contraction and show almost complete absence of response to the second stimulation. For extinction in the waking state the optimal interval was found to be 4 seconds.

We now turn to the description of the effect of the physical character-

istics of the stimulation upon extinction. In this paper we shall consider only those experiments in which the two stimulations of a pair were identical. This is the procedure to which we shall refer in this connection as experiments under "standard" conditions.

II. *The physical characteristics of the stimulation.* These are: 1, its duration; 2, its pattern-frequency or the number of pulses per second; 3, its pulse-frequency or the inverse of the pulse duration; 4, its voltage.

1. *The duration of the stimulation.* Extinction has been found to be more pronounced with longer stimulation, less with shorter. Figure 8 shows four pairs of stimulations, all identical in every respect except for the duration in each pair. The first pair shows marked facilitation of the

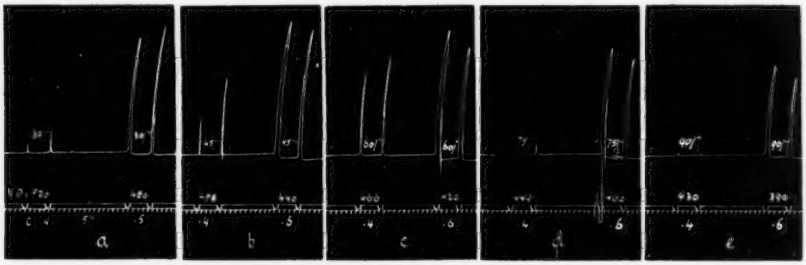

Fig. 10. Each record in this figure shows 2 pairs of stimulations. The stimulations of each pair were identical; the 2 pairs differed in the pulse-frequencies and voltages used. The change in voltage in each record was made so as to give exactly equal stimulation voltage. First pair in each record with higher pulse-frequency, i.e., shorter pulses (smaller capacity, 0.4 microfarad in circuit), second pair with lower pulse-frequency, i.e., longer pulses (larger capacity, 0.6 microfarad in circuit). In record *a* low pattern-frequency (30 per sec.) results in facilitation with the two pulse-frequencies. In records *b*, *c* and *d* with pattern-frequencies of 45, 60 and 75 per second respectively, facilitation is apparent with the higher pulse-frequency, extinction with the lower pulse-frequency. Record *e*, taken at a pattern-frequency of 90 per second, shows extinction with both pulse-frequencies.

second response with a short stimulation (2 sec.), the second evidences less facilitation with stimulation of 2.5 seconds. In the third pair with stimulations of 3 seconds the two responses are equal, whereas the fourth pair shows extinction with stimulations of 3.5 seconds' duration.

2. *The pattern-frequency of the stimulation.* Higher pattern-frequency produces more, lower pattern-frequency less extinction. In figure 9 six pairs of stimulations are shown. All pairs of stimulations were identical with respect to interval, duration and pulse-frequency and all but the last with respect to voltage. In the first record the pattern-frequency of 114 pulses per second results in partial extinction of the second response. In the second record with 65 pulses per second very slight extinction is mani-

fest. In records 3 and 4 with pattern-frequencies of 53 and 44 respectively the two responses in each pair are approximately equal. In record 5 with a pattern-frequency of 36 there is unmistakable facilitation of the second response. This is still more marked in record 6, where a pattern-frequency of 21 was used. To obtain any response with this low frequency the strength of the stimulation had to be increased to 8000; this record, therefore, is not strictly comparable to the other five, but shows the same thing.

3. *The pulse-frequency of the stimulation.* The pulse-frequency is also of importance. It was found that lower pulse-frequency (i.e., longer pulses) produces more, higher pulse-frequency (i.e., shorter pulses) less extinction. Figure 10 demonstrates the influence of this characteristic conclusively. In all six records the voltage-divider was set to give exactly equal voltages as measured on the cathode ray oscillograph with the animal in the circuit (12). The durations and intervals were the same throughout; the only change in any record was in the capacities used, being larger (i.e., working

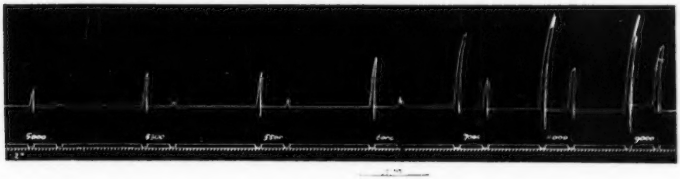

Fig. 11. Increase of extinction, as measured by the difference in size of the two responses of each pair, with increase of voltage of stimulation.

with longer pulses) in the second pair than in the first of each record. Records *b*, *c* and *d* show facilitation with the shorter, extinction with the longer pulses. In record *a* the low pattern-frequency used prevents extinction, in record *e* the high pattern-frequency precludes facilitation.

The corresponding pairs in these records present another example of the influence of pattern-frequency alone, namely, the reversal of facilitation to extinction with increase of this frequency. It will be noted that with the smaller capacity (0.4 μ F) the reversal occurs at a higher pattern-frequency than with the larger capacity (0.6 μ F), thus demonstrating in another way the influence of pulse-frequency on extinction.

4. *The voltage of the stimulation.* The voltage of the stimulations has also a great influence on extinction. If the two stimulations are slightly supraliminal, the second response is usually absent; with stronger stimulations it is usually small. This is illustrated step by step in figure 11. Thus it seems as if two competitive factors operate to determine the size of the response to the second stimulation: 1, the extinguishing factor

"hanging over" in the cortex from the first stimulation; 2, the excitation set up by the second stimulation. With weak stimulations the extinguishing factor dominates; with stronger stimulations the excitatory process overcomes to some extent the extinction. That this latter factor is operative is shown by the fact that the second response of each pair is smaller than the first response.

Extinction, i.e., the difference in size of the two responses, increases with increase in voltage. This is evident from figure 11, especially from the first four pairs of responses. In the last three pairs this feature is obscured to some extent by the slow speed of the paper. Interesting in this respect is figure 12, where due to the fairly low pattern-frequency used and the other conditions of the experiment the first three pairs of stimulations gave facilitation, the last two pairs increasing extinction with increase of the voltage of stimulation.

Figures 6, 8, 9, 10, 12 are of importance in that they indicate how facilitation complicates extinction. Other experiments, which will be dealt with

Fig. 12. Decrease of facilitation and reversal into extinction with increase of voltage of stimulation. That in the first three pairs facilitation appears is mainly due to the relatively low pattern-frequency (47 per sec.) used.

in another paper, have shown that a factor for facilitation is operative for a much longer time than is evidenced in the extinction experiments of this paper. It is only because of the rigid control of the experimental conditions, above all of stimulation, that this factor for facilitation does not prevent analysis of the conditions determining extinction.

Summarizing the evidence presented, it has been shown that under "standard" conditions of stimulation, given a certain physiological condition of the cortex and a certain interval, the four physical characteristics of stimulation determine its efficacy in producing extinction.

When these four characteristics are all held constant, the results are constant. When any one is changed, so as to increase or decrease the total energy of stimulation, extinction is increased or decreased respectively. When two or more are changed in the same direction, extinction is changed correspondingly.

However, when two of the characteristics are changed in opposite directions, even though the total energy be kept constant, extinction may alter in either direction, depending upon the values of all four characteristics.

Therefore, given the physiological condition of the cortex and the interval of the two stimulations, extinction is not a function of the total energy of stimulation only, but depends upon the particular value of each of the four characteristics entering into its composition.

But even this is not the whole story. Experiments with pairs of *unequal* stimulations have shown that the change in size of the response to the second stimulation is determined not only by the characteristics of the first stimulation, but also by each and every one of the characteristics of the second stimulation.

We reserve the presentation of the findings of such experiments for another paper, but wish to make one exception of importance here, namely, the observation that extinction can be obtained with subliminal antecedent stimulation. In the five records of figure 13 all stimulations are identical except with respect to the voltage of the first stimulations of each pair. Record 1 is given to show complete extinction appearing under "standard"

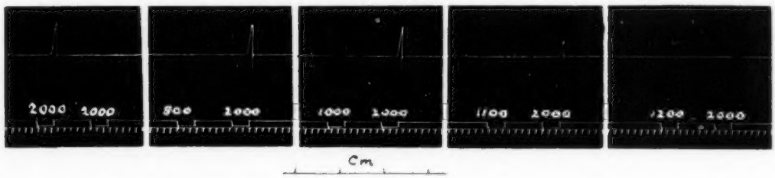

Fig. 13. This figure shows extinction with subliminal antecedent stimulation. First record complete extinction under "standard" conditions. Antecedent stimulation at voltage 800 does not affect second response. Subliminal stimulations of 1000, 1100 and 1200 produce increasing extinction.

conditions; the subsequent records show increase of extinction with increase of voltage of the first stimulation from 800, where there is no extinction, to 1200, where it is almost complete. The first stimulations in these last four records are all subliminal, in fact the first is ineffective.

Discussion. Extinction should be distinguished from inhibition. Inhibition in physiology of the cortex is, as far as it is known at present, the disappearance of an existing muscular contraction on stimulation of a cortical focus from which an antagonistic response may be elicited. Extinction is the diminution or absence of response to stimulation of a motor focus following antecedent stimulation of that same focus. Inasmuch as it has been shown that extinction originates in the deeper layers of the cortex, subjacent to the electrode(s), it follows that during extinction the motor focus stimulated is temporarily inactivated. It should be understood that in distinguishing between inhibition and extinction, it is not implied that the two phenomena may not be fundamentally related in some way. The nature of this inactivation is such that extinction cannot be

explained on the basis of fatigue or exhaustion of the focus stimulated. This statement is established by the following facts. In the first place, extinction is apparent after weak and even after subliminal stimulation. Secondly, if the two stimulations are applied with an interval of a few seconds only, it is found that the second response is markedly facilitated. Thirdly, during extinction facilitation is obtainable. This can be demonstrated if two equal stimulations at a facilitating interval of two or three seconds are applied so that both fall within the period of extinction of an antecedent, identical stimulation; it is found then that the response to the last stimulation is always larger than that to the second and may even be larger than that to the first stimulation (6).

The nerve cells of the cerebral cortex, like any other nerve cells, must have a refractory period. In fact, Broca and Richet (2) have stated that the refractory period of the motor cortex of dogs under chloralose anesthesia is 0.1 of a second. It is not quite certain whether their period represents a real refractory period, for they state that *within* their refractory period lies a short "période d'addition," which is of the order of hundredths of seconds. Unfortunately their figure is the only one available for the cortex. Probably the true refractory period of the efferent moto-neurones is much less, for later figures on the absolute refractory period of moto-neurones in the spinal cord (Eccles, 7) and in the brain stem (Lorente de Nó, 14) are much lower, 2.4 and 0.6 sigma respectively. But even if one takes the larger figure of Broca and Richet (100 sigmas), thus strengthening the argument, it is clear that, given the discrepancy between its duration (0.1 sec.) and the optimal interval for extinction (13 sec. or more), such a "refractory period" cannot be identified with the local stimulatory inactivation of the deeper layers of the cortical focus, which is the factor for extinction.

Neither can extinction be a manifestation of a factor of polarization of the tissue, since it remains unaltered on reversing the polarity of half-rectified pulses (thyratron, 60-cycle A.C.) and is regularly elicitable with 60-cycle A.C. Moreover, this possibility is excluded by the fact that the same amount of extinction is produced by an antecedent stimulation so long that the response to it has ceased before the end of stimulation, as by one twice as long. This shows that extinction is due to inactivation of the nerve cells in consequence of their repeated discharge, and not to mere transmission of the stimulating current through the cortex.

It was established by the method of Lane, Prescott and the writers (13) that the voltage and current pictures on the cathode ray oscillograph of both equal stimulations in any pair were identical, whereas the voltage of the second stimulation had to be increased by at least 10 to 20 per cent to overcome extinction and elicit a second response of the same size as the first. These findings show that extinction is not due to differences in the physical characteristics of the two stimulations.

Two possible explanations of extinction remain for consideration. Lorente de Nó has suggested, both in discussion and by personal communication, that the diminution or absence of response to the second stimulation results from an absence of background of activity in reverberating circuits, because they have been forced to discharge simultaneously and therefore have become simultaneously refractory.

The other explanation, in analogy with the findings in peripheral nerves (12, 9, 10), is that repetitive stimulation results in a cumulative physicochemical change manifest as a positive after-potential and associated with a decrease in excitability. The time-relations of these positive after-potentials in peripheral nerves and their accompanying changes in threshold are comparable to the time-relations for extinction. Findings suggesting that both of these explanations may be to some extent applicable to the problem of extinction will be discussed in another paper.

Whatever the explanation for extinction may be, we wish in conclusion to point out that the experiments presented in this paper show that under "standard" conditions it is possible to predict the change in size of the second response, when anyone of the four physical characteristics of stimulation is changed. The reproducibility of these findings under the conditions of our experiments indicates that these characteristics are the chief, if not the only significant variables determining extinction.

SUMMARY

1. Extinction is the diminution or absence of response to a stimulation of a focus of the motor cortex following an antecedent stimulation of that same focus at a suitable interval.

2. Extinction is a cortical phenomenon, a local stimulatory inactivation of the deeper layers of the cortex, probably of the large and giant pyramidal cells.

3. The phenomenon and the interval at which it is manifest are dependent upon the physiological condition of the cortex, above all upon the depth of anesthesia and the circulation. In the non-narcotized monkey the optimal interval for extinction is 4 seconds, under moderately deep narcosis 13 to 15 seconds, in deep anesthesia 1 to 3 minutes.

4. Extinction is further influenced by the physical characteristics of the stimulations, being dependent upon *a*, the duration; *b*, the pattern-frequency; *c*, the pulse-frequency; *d*, the voltage of stimulation. Extinction is increased or decreased when the total energy of stimulation is augmented or diminished by an appropriate change in any one of these four characteristics. Yet it is not a function of the total energy of the stimulation only.

5. Extinction is apparent after antecedent subliminal stimulation.

6. The reasons for establishing extinction as a distinct phenomenon,

apart from inhibition, fatigue, refractory period are set forth. It is not due to physical or physiological polarization.

7. Two possible explanations are indicated.

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THE EFFECT OF ADRENALECTOMY ON LIVER FAT IN FASTING AND AFTER THE ADMINISTRATION OF ANTERIOR PITUITARY EXTRACTS

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Certain extracts of the anterior pituitary gland produce a ketosis in fasting animals (1, 2). It has been shown rather definitely that the ketone bodies are formed in the liver (3) and Mirsky has found (4) in liverless animals that ketogenic anterior pituitary extracts fail to increase the ketone bodies in the blood. This suggests a relation between the ketosis which occurs and the deposition of fat in the liver which results from the administration of anterior pituitary extracts (5, 6). The ketosis is prevented by removal of the adrenals (7). These experiments were carried out to determine the influence of adrenalectomy upon the deposition of fat in the liver which is caused by the extract.

Typical results are presented in table 1. The rats were operated upon and then fasted for four days. Anterior pituitary extract was prepared according to the procedure of Best and Campbell (6, p. 191) from a commercial (Wilson Laboratories) anterior pituitary powder. The male groups receiving the extract were given 3 cc. subcutaneously each day while the females were given 5 cc. daily of an old and weaker but still active extract. Liver fat was not determined as such. The fatty acids and nonsaponifiable lipids which are a good measure of fat content were determined by alkaline digestion of the organ with resulting saponification of the fat and extraction for weighing after acidification.

The data show clearly that removal of the adrenals prevents the deposition of the fat in the liver just as this procedure prevents the ketosis (7) when ketogenic anterior pituitary extracts are used. It is interesting that adrenalectomy has also been found (8) to prevent the fatty infiltration of the liver which usually accompanies total pancreatectomy. An examination of the groups in table 1 which did not receive pituitary extract shows that adrenalectomy also reduced the accumulation of fat in the liver during the four days of fasting.

The increase in the size of the livers in the normal rats receiving the anterior pituitary extract has been noted before (6). Such increase in liver weight as occurred in the present experiments was just as great in the

TABLE 1

NUMBER	CONTROLS							ADRENALECTOMIZED						
	Body weight		Body surface	Liver weight		Liver fat		Body weight		Body surface	Liver weight		Liver fat	
	Initial	Death		Actual	Per 100 sq. cm. B. S.	Per cent	Mgm. per 100 sq. cm. B. S.	Initial	Death		Actual	Per 100 sq. cm. B. S.	Per cent	Mgm. per 100 sq. cm. B. S.
	grams	*grams*	*sq. cm.*	*grams*	*grams*			*grams*	*grams*	*sq. cm.*	*grams*	*grams*		
Experiment 1. Males														
Rats receiving no extract														
1	225	195	420	5.18	1.24	5.34	66	204	177	394	5.33	1.35	2.95	40
2	205	178	395	4.34	1.10	4.78	53	203	161	392	4.32	1.10	2.54	28
3	236	198	433	5.58	1.29	2.57	33	198	168	386	4.45	1.15	2.52	29
Average..	222	190	416	5.03	1.21	4.23	51	202	169	390	4.70	1.20	2.67	32
Rats to which anterior pituitary extract was administered														
4	222	186	416	6.02	1.45	12.60	181	240	207	437	5.78	1.32	2.92	38
5	227	195	422	5.62	1.33	12.20	163	220	198	413	6.14	1.49	2.78	41
6	238	211	435	6.25	1.44	7.75	110	208	190	375	4.93	1.31	2.76	37
Average..	229	197	424	5.96	1.40	10.85	151	223	199	408	5.62	1.37	2.82	39
Experiment 2. Females														
Rats receiving no extract														
1	171	155	351	4.65	1.32	5.26	70	163	143	338	4.35	1.29	4.16	53
2	160	130	334	3.75	1.12	4.92	55	180	149	362	4.60	1.27	2.81	36
3	149	127	318	3.96	1.24	3.55	44	157	140	330	3.95	1.20	2.74	33
4	151	143	322	3.62	1.12	3.24	36	169	138	346	4.24	1.22	2.58	32
5	172	149	351	5.00	1.43	2.45	35	149	125	318	3.78	1.19	2.32	27
Average..	160	141	335	4.19	1.24	3.88	48	163	139	339	4.18	1.23	2.92	36
Rats to which anterior pituitary extract was administered														
6	163	140	338	6.30	1.86	12.20	227	155	142	327	4.95	1.51	2.77	42
7	180	160	362	7.05	1.95	6.43	125	151	143	322	4.98	1.55	2.35	36
8	167	151	344	6.10	1.77	6.30	112	163	154	338	5.50	1.62	2.12	34
9	149	138	318	5.90	1.85	5.00	93	182	171	365	5.66	1.55	1.87	29
10	153	134	325	5.85	1.80	4.80	86	163	150	338	4.50	1.33	1.75	23
Average..	162	145	337	6.24	1.85	6.95	128	163	152	338	5.11	1.51	2.17	33

* Body surface calculated from body weight before fasting by the formula B. S. = (B. W.) 2/3 × 11.36.

adrenalectomized rats as in the controls, although in the former the liver fat was not elevated by the pituitary extract.

The ketogenic anterior pituitary extract produces a greater ketosis in female than in male rats (7). Campbell has reported (9) this sex difference in the liver fat accumulation following the administration of the extract to guinea pigs. We have observed the latter in rats. It is not present in the experiments given here because the two sexes did not receive similar quantities of extract.

SUMMARY

Anterior pituitary extracts which have the property of producing a ketosis in fasting rats also cause a deposition of fat in the liver. Adrenalectomy not only abolishes the ketosis but prevents the accumulation of fat in the liver. Removal of the adrenal glands also reduces the amount of fat which is deposited in the liver during fasting.

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THE RETROGRESSION OF THE LACTATING MAMMARY GLAND IN THE GUINEA PIG[1]

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Kuramitsu and Loeb (1) tested in guinea pigs the effect of suckling in one breast on the course of involution in another breast, in which suckling had been made impossible by a ligature placed around the nipples soon after labor; it was found that the gland which as a result of ligation could no longer be used for suckling, retrogressed rapidly in a way comparable to the involution of the mammary glands in the non-nursing guinea pig, whereas the suckled gland continued to be large and to produce milk. It was concluded, therefore, that it is solely the local effect of the withdrawal of milk which maintains the active state of lactation in the mammary gland and that reflexes emanating from the suckled gland are not of importance in maintaining the secretion of the mammary glands. Involution of the non-suckled mammary gland of one side would then occur without regard to the function or lack of function in the gland of the other side. In the involuting gland, the size and number of acini and lobules decrease, the connective tissue becomes more prominent and certain degenerative processes may occur in the acinus cells. In agreement with these observations were the results obtained by Hammond in the rabbit (2). However, subsequently Selye (3) found in the rat that if all the main milk ducts were cut three days after labor and the young were allowed to suckle, the alveoli of the mammary glands were still well preserved and filled with milk as late as 14 days following the operation, while in control rats, in which suckling had been prevented, the process of involution was marked about one week after removal of the young. Likewise if cuts were made through the milk ducts of the mammary glands on one side, which made possible the continued suckling on this side, and if at the same time the nipples on the other side were excised and thus suckling was prevented here, milk production continued in all the mammary glands, including those in which the nipples had been excised. This indicated that it was due to a nervous mechanism set to work by suckling in some of the glands, that the normal

[1] These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

milk secretion was maintained also in the gland in which suckling had been prevented. These conditions applied to the rat. In the goat, Turner and Reineke (4) found that if milking is suspended 30 days after labor the formerly secreting alveoli of the mammary glands degenerate, and 75 days after the last milking only a duct system remains. When in the goat suckling is stopped on one side, but is allowed to continue on the other side, involution is retarded in the not-milked side. But if at a relatively late stage of lactation suckling was prevented on one side stimulation of the mammary gland of the other side did not seem to transmit a secretory stimulus to the not-milked side, whereas at earlier stages such a process occurred. At later stages following labor the retrogression of the mammary gland had become a locally determined process which could not be prevented by nervous stimuli.

Following the experiments of Selye it has been assumed by several writers that the conditions obtaining in the rat applied generally to mammals and that secretion in the mammary gland could in all cases be maintained by means of reflexes.

In view of the results obtained previously by Kuramitsu and Loeb in the guinea pig, which were not in accord with such a conclusion, we decided to undertake a new series of experiments in guinea pigs in order to determine whether species differences exist in regard to the factors which determine secretion and involution in the mammary gland. In this new series, we used altogether 25 guinea pigs in which pregnancy had been determined. In ten of these animals nursing was allowed to proceed without interference; in four others the young were removed within a few hours following labor, in order to prevent suckling and in a third series of 11 guinea pigs, the nipple of one breast was ligated soon after labor, while the breast of the other side was not interfered with. In some of these animals the ligated nipples were removed by cutting. At the conclusion of the experiment, pieces from the ligated and non-ligated mammary glands were studied in each case microscopically. Our findings were as follows:

If suckling has ceased normally at the time when the young have reached a state of maturity and have become independent of the mother's milk or if it is interrupted prematurely as a result of ligation of the ducts or of an early removal of the young, the mammary gland assumes a typical structure, characterized by a shrinking of the acini and their component cells and by certain degenerative conditions in the lobules and acini. The signs of secretion decrease markedly, the acini and lobules diminish in number and there is a relative or absolute increase in connective tissue. However, even in such a retrogressing gland there may still be seen some areas where the acini are large, resembling those which are found in the secreting gland; they are noted especially in the periphery of the gland or around some larger ducts, in places where presumably the circulation is especially active.

If soon after labor the milk ducts of one of the mammary glands were ligated and thus the emptying of the ducts was prevented, while the gland of the other side was left intact and could therefore be used for suckling, a marked difference developed within a short time, not later than a few days, between the breasts on the ligated and non-ligated side. The latter behaved like a nursing gland, while the former behaved like an inactive, retrogressing gland. However, certain irregularities occurred here in the ligated and non-ligated breasts which we found also in the case of the ordinary nursing and retrogressing glands. It is possible that the interference with suckling in one breast made the full use of the other gland somewhat more difficult, and somewhat increased in the latter the amount of the resting acinus tissue; but the difference from the nursing gland in the normal guinea pig, not subjected to any experimental interference, was so slight, that we may have to deal merely with accidental variations.

Our experiments confirm then the results which Kuramitsu and Loeb had obtained previously and we may conclude that in the guinea pig the secretion of the mammary gland following the completion of the state of pregnancy is maintained by the suckling of the gland and by the removal of the secretion and that if the secretion is not removed retrogressive changes set in irrespective of the state of the other breast. Reflex stimulations transmitted from the suckling gland to the ligated gland, in which the secretion stagnates, are not of marked significance in the guinea pig. It is a local effect which determines in the latter species the state of the mammary gland. If results are different in the mammary gland of the rat, we must consider that conditions under which the mammary glands function differ in these two species. In the guinea pig only two mammary glands are present, while in the rat their number is larger. Furthermore, in the rat the young depend upon the mother's milk a much longer time than in the guinea pig; correspondingly the secretion of the mammary gland is more adequately secured in the rat than in the guinea pig.

While the mechanisms controlling the sexual cycle are in principle the same in all mammals, there are considerable variations in different species in many component parts of these mechanisms. To mention only one very striking difference, which has been well established, we may refer to the difference in the effect of hysterectomy in guinea pig, rat and rabbit, as far as the preservation of the corpus luteum and the duration of the sexual cycle are concerned. In general in each species the different component parts of the cycle are fitted together in such a fashion that the sex functions of the whole organism are most efficiently maintained.

CONCLUSION

Ligation of the nipple of one of the breasts following labor in the guinea pig is followed by a rapid onset of retrogressive changes in this mammary

gland, while the mammary gland of the other, non-ligated side may continue to secrete milk in an approximately normal manner, if suckling proceeds on this side. In the guinea pig, secretion and retrogression of the mammary gland following pregnancy are therefore controlled essentially by local mechanisms, in contrast to the mode of regulation which has been observed in the rat.

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OLFACTORY AND TRIGEMINAL CONDITIONED REFLEXES IN DOGS

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The purpose of this study is first, to determine what vapors can be relied upon to elicit a conditioned reflex only over the olfactory nerves in dogs, and second, to see if the same vapors which alter respiration over the olfactory or trigeminal nerves are capable of evoking conditioned responses over the same nerves.

PROCEDURE. Obviously the complete technique used in 1928 for producing an "olfactory" or "trigeminal" animal cannot be used in this problem. The dogs used for testing the olfactory conditioned reflexes had their maxillary and naso-ciliary nerves cut in orbits.[1] Those used for the trigeminal reflexes had their olfactory bulbs transected, accomplished by a dorsal exposure through the frontal sinuses.[2] Either procedure limited stimulation to the nerves in question as shown by a series of experiments in which conditioned reflexes could not be induced and respiration was unaltered when both of these procedures were carried out on the same dog and inhalations of the vapors were held to the same concentrations and for the same duration of time as used in this study.

Since it was not necessary to a make quantitative study of these conditioned reflexes the writer used a slight modification of the method James used in his investigations on the sound reflex. The unconditioned stimulus was the same, namely, an electrode attached to a foreleg. The problem for the dog was to avoid a shock by raising his leg in response to a nasal stimulation from a vapor presented 7 seconds before shocking. This movement of the leg prevented the shock by a pull on a string which connected the foreleg to a wall switch.

The tests were made in sound proof "furtex" rooms after Pavlov's construction. The recording apparatus was placed on a table in the experimenter's room. Two sets of wires passed through the partition into the dog room. Circuit 1 starting from dry cells leads to a switch in front of

[1] The central stumps of the maxillary nerves were turned back and tied to eye muscles to prevent regeneration.

[2] The sodium amytal used for anesthesia on all adult dogs was generously donated by the Eli Lilly Co.

the experimenter, to an inductorium, signal magnet for kymograph and wall switch in dog room. Circuit 2 goes from inductorium to a second switch in front of experimenter to electrodes attached to dog's foreleg. After adding time marker to trace seconds, a complete record was readily obtained as follows: Closing switch 1 with the presentation of the vapor produced a downward stroke of the signal magnet. A positive response of the dog's leg from the vapor was indicated by an upstroke of the signal magnet and was marked (+). A shock for failure to respond to a vapor also produced an upstroke of the signal magnet and was marked (0) to distinguish it from a positive response.

The dogs were either blinded or blindfolded and the vapors were usually presented by an assistant, from wide-mouthed bottles, the distance from the nostrils depending on the volatility of the vapors. An empty bottle was frequently substituted for controls. One or more conditioned responses were always obtained from each dog by introducing the stimulating vapor into a current of air which passed directly in front of the dog's head. This current of air terminated in a small rubber coil having a single row of holes punched along its inner margin. With this coil attached in front of of the dog's muzzle and vertical to his snout, it was immaterial how much the dogs moved, the vapor from the coil would be inhaled. The chief objection to this procedure and which prevented it from being used throughout was that considerable time was required to free the room of the large amount of vapor pumped into it during a test. The constant noise from the running motor and air pump in no way interfered with the tests. In fact most dogs preferred the hum to the absolute quiet and were less nervous. In addition the hum made inaudible any noise from the recording instruments or other sources. The air pump also improved the ventilation and was run continuously throughout all of the later tests.

In 30 normal or "olfactory" dogs and 6 "trigeminal" dogs it ordinarily required 15 to 25 trials before the animal first raised his leg in response to a vapor warning. One responded on the third trial, while a very stupid dog required 41 trials and did not react regularly until the 60th test. Most dogs acquired the reflex quickly after the first genuine response with only an occasional failure. Once having acquired the reflex many dogs would react positively to 25 tests of a vapor and negatively to numerous empty bottle controls a day for two or even more successive days, but like all conditioned reflexes this one is extinguished in time if not occasionally reinforced by the unconditioned reflex. It seldom required more than 6 seconds for a response, usually 1 to 3, so that failure to react in 7 seconds was followed by a shock. Exceptions, however, were made for the controls and for the first trials of a new and different vapor. By a response is meant an actual lifting of the foreleg from vapor stimulation that is identical in every respect to the unconditioned response. If the dog was the

type that growled or barked with the shock stimulation he would do likewise with the vapor stimulation. The intervals between tests varied from 2 to 10 minutes depending on the temperament of the dog.

TABLE 1

DATE OF TESTS	CLOVES	LAVENDER	ANISE	ASAFETIDA	BENZOL	XYLOL	EUCALYPTUS	CAMPHOR	BUTYRIC ACID	PYRIDIN	CHLOROFORM	PHENOL	CONTROL BLANKS
Apl. 8	8 = + 1 to 6″	2 = + 2 & 4″		3 = + 3 to 6″									
	22 = 0*			1 = 0 12″									6 = 0 15″
Apl. 10	10 = +** 1 to 4″												
	2 − 0** 7″												
Apl. 14	6 = + 2 to 6″	3 = + 4 to 7″	2 = + 3 & 7″	1 = + 4″	2 = + 2 & 6″			2 = + 4″	2 = + 4 & 6″		3 = + 3 to 5″	2 = + 4 & 7″	1 = +‡ 2″
	1 = 0 7″	1 = 0 7″	1 = 0 8″	2 = 0† 10″			1 = 0 8″	1 = 0 8″	1 = 0 8″			1 = 0 8″	7 = 0 15″
Apl. 20	3 = + 2 to 4″				1 = + 2″		5 = + 4 to 7″	8 = +		1 = + 8″	6 = + 3 to 7″		
							3 = 0 10″	2 = 0 7 & 9″		1 = 0 10″	1 = 0 8″		7 = 0 15″
May 1	13 = + 1 to 5″		5 = + 4 to 7″	7 = + 2 to 8″									1 = +‡ 6″
	6		1 = 0 8″	2 = 0 8 & 9″									7 = 0 15″
May 3	9 = + 1 to 6″	5 = + 2 to 7″	3 = + 3 to 7″										
	1 = 0 7″	2 = 0 8″											6 − 0 15″
May 10			2 = + 2 to 4″			4 = + 2 to 6″			3 = + 3 to 5″	6 = + 2 to 6″			
						1 = 0 8″			2 = 0 7″	1 = 0 7″			6 = 0 15″

* Includes the first tests which are always negative.
** Air current procedure was used for all stimulations.
† No shock followed these negative responses.
‡ Switch broken by general body movement and should be regarded as negative.

An occasional dog had periods when he broke the switch from general nervous movements and some dogs that had been shocked many times would keep raising the leg for short intervals so that it was difficult to

secure a control record. When such conditions developed it was necessary to wait for a quiet period, to test a vapor immediately after one of these movements or in some instances to tie the dog's hind legs to a pole so that his weight was thrown on his forelegs.

All dogs were autopsied to make certain that the olfactory bulbs had been completely transected or that the maxillary and naso-ciliary nerves had not regenerated.

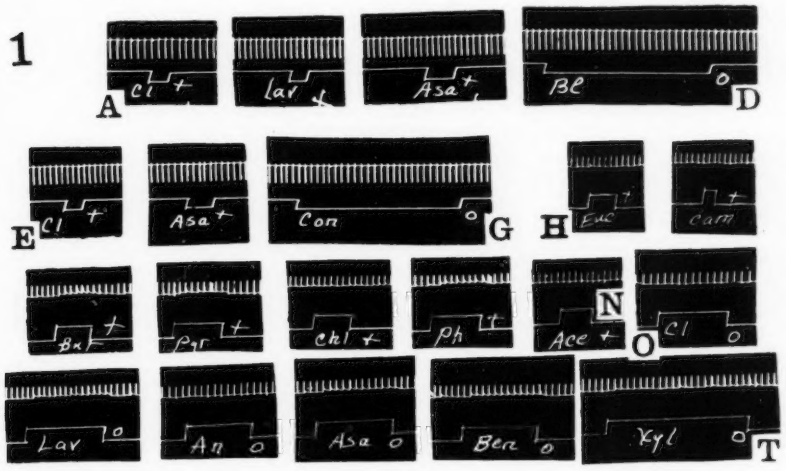

Fig. 1. Upper tracing, time in seconds; lower tracing, first break in line, signal for presenting vapor, second break, positive (+) or negative (0) response.

A to D, positive conditioned responses from cloves, lavender, asafetida and a negative control over the olfactory nerves from a blinded dog. E to G, positive conditioned responses from cloves, asafetida and a negative control from a blinded dog which had olfactory bulbs severed later. H to N, positive conditioned responses from eucalyptus, camphor, butyric acid, pyridin, chloroform, phenol, acetic acid, and O to T, negative responses from cloves, lavender, anise, asafetida, benzol and xylol from above mentioned dog after olfactory bulbs had been transected.

Olfactory conditioned reflex. Numerous tests from a typical dog in which the maxillary and naso-ciliary nerves were cut are given in table 1. In this table the upper horizontal column opposite any date shows the number of positive responses (+) from the vapors used and the minimum and maximum time in seconds (″) required for these responses; while the number of negative responses, if any, appear in the column below.

Figure 1, A to D, shows the first positive responses for cloves, lavender, asafetida and a negative control. As a result of these olfactory tests on this partially trigeminized dog and five others it can be stated that any of the vapors listed in the previous table can evoke an olfactory conditioned reflex of the foreleg. All of these dogs yielded some positive conditioned

reflexes from inhalation of formalin, acetic acid and ammonia from bottles held 6 inches from the nostrils for shorter intervals than was required to elicit a vagal reflex affecting respiration. These responses were presumably

TABLE 2

DATE OF TEST	CLOVES	LAVENDER	ANISE	ASAFETIDA	BENZOL	XYLOL	EUCALYPTUS	CAMPHOR	BUTYRIC ACID	PYRIDIN	CHLOROFORM	PHENOL	ACETIC ACID
Apl. 23													5 = + 2 to 7″
	2 = 0 7″	6 = 0 7″											19 = 0* 7″
May 1							16 = + 2 to 6″					4 = + 2 to 6″	4 = + 1 to 4″
	5 = 0 7″				3 = 0 7″		4 = 0 7″						
May 10								6 = + 1 to 5″			4 = + 2 to 5″		5 = + 1 to 3″
				4 = 0 7″	4 = 0 7″	5 = 0 7″		1 = 0 7″			1 = 0 7″		
May 12									8 = + 2 to 5″	3 = + 2 to 4″	2 = + 2 to 5″	2 = + 3 to 5″	
		3 = 0 7″	4 = 0 7″	3 = 0 7″		2 = 0 7″			2 = 0 7″				
May 14								2 = + 2 to 4″		3 = + 2 to 3″	4 = + 2 to 5″		
	6 = 0 7″	5 = 0 7″	8 = 0 7″	9 = 0 7″	2 = 0 7″					1 = 0 7″			
May 18									3 = + 2 to 5″			Sheep dip 3 = + 2 to 6″	7 = + 1 to 3″
	4 = 0 7″	4 = 0 7″		3 = 0 7″	5 = 0 7″	3 = 0 7″			1 = 0 7″				
May 20								5 = + 2 to 5″	2 = + 3 to 4″		7 = + 2 to 4″		
	10 = 0 7″	3 = 0 7″	4 = 0 7″								1 = 0 7″		

* Includes the first tests which are always negative.

olfactory in origin, but since the IX and X nerves were not eliminated it seems advisable not to include these strong vagal irritants as vapors which can induce an olfactory conditioned reflex.

It is obvious from this table that there are more negative responses from other vapor than for cloves after the animal had been conditioned to cloves.

During very light barbital anesthesia or no anesthesia, respiration was depressed and generally slowed in these partially trigeminized dogs during inhalation of lavender, cloves, anise, wintergreen, benzol, xylol, asafetida, butyric acid, pyridin, camphor, eucalyptus, ether, chloroform, phenol and sheep dip. Respiration was also considerably depressed from insufflation of formalin, acetic acid and ammonia into the nostrils after cannulae had been inserted into their trachea and pharynx.

Observations on these dogs demonstrated how much a dog depends on his snout for a tactile organ. A blinded dog with a trigeminized snout is greatly handicapped over a blinded dog in ascending a flight of stairs. A blinded dog with a denervated snout experiences great difficulty in eating soft food from a pan. He will pack the food downward and to one side by pounding it with his snout, leaving a large rim of food around the bottom of the pan. One dog gradually reduced the length of his snout by one half through pawing food from his nostrils.

Trigeminal conditioned reflex. The dog selected to typify 5 blinded anosmic dogs was chosen because of the rapidity with which he first responded to cloves (7th trial) before his olfactory bulbs were severed. The following positive tracings for cloves, asafetida and the negative control (fig. 1, E to G) were recorded before the bulb operation.

The results of numerous conditioned vapor tests on this anosmic dog[3] are summarized in table 2 after a similar arrangement used for the olfactory tests. In these trigeminal tests a sufficient number of non-responsive vapors were used in each experiment to serve for controls so that it was not necessary to take any records from empty bottles.

It is clear from this table and from the tracings (fig. 1, H to N) that eucalyptus, camphor, butyric acid, pyridin, chloroform, phenol and acetic acid can be used for trigeminal irritants for eliciting conditioned reflexes in anosmic dogs provided they are not used long enough or in sufficient concentrations to act over the IX and X nerves. It is also obvious from this table and figure 1 (O to T), that cloves, lavender, anise, asafetida, benzol and xylol, previously shown to be unexcelled for inducing an olfactory conditioned reflex, do not evoke a trigeminal conditioned response.

An experiment was made in this animal before the olfactory bulbs were severed to determine the number of trials it would require to establish this conditioned reflex on the contralateral leg. On April 16 the left foreleg

[3] This operation was performed on April 16, 1936. Three days later and throughout life this blinded anosmic dog was unable to detect ground meat when placed close to his nostril and could not select a paper package of ground meat from 3 paper packages of like size. The dog would go up and down stairs quite rapidly using his snout as a tactile organ for judging distances between steps.

was substituted for the right. After delivering a few preliminary shocks the first response to cloves came with the fifth trial in 3 seconds. This response, however, was preceded by one from the right leg. In each of the first three tests the right leg was raised as soon as the odor was perceived and the dog was shocked in 7 seconds for failure to move his left leg. The fourth trial was negative for both legs and shock followed in 7 seconds. The 6th to the 8th tests were positive, but came from a general movement that involved both legs. After a 20 minute interval and shock, the dog was able to respond with only the left foreleg to 4 trials of cloves in 1 to 2 seconds and to 2 tests of asafetida in 3 and 5 seconds. Four negative empty bottle tests for 15 seconds each served as controls.

The inhalation-respiratory reflex was tested in this anosmic dog on April 22, under no anesthesia and under light barbital, with the result that respiration was depressed from inhalation of camphor, eucalyptus, ether, chloroform, butyric acid, pyridin, phenol and sheep dip, but was unaltered from cloves, lavender, anise, wintergreen, asafetida, benzol or xylol.

Trigeminal conditioned reflexes in a dog in which the eyes were enucleated and the olfactory bulbs were severed at birth. This procedure was attempted on several pups which were less than a day old to determine if the trigeminal fibers of the nasal cavities, in absence of olfactory and optic impulses, would ultimately acquire more or less sensitivity to a purely olfactory stimulus. Unfortunately most of these pups were unable or could not be induced to suckle and only two became adults. Autopsy demonstrated both olfactory bulbs completely severed in one of these dogs and one bulb completely and one bulb partially severed in the other dog. It was impossible to elicit conditioned responses from cloves, lavender, anise, asafetida, xylol and benzol from the dog in which both bulbs had been completely transected. Also this dog was unable to select a paper package of ground meat from three paper packages of like size when placed in a pan or on the floor or when suspended from 4 rods. In fact this dog would not follow a piece of meat when passed directly below his nostrils, nor did he exhibit any excitement when a female dog was placed in the same room.

Discussion. The literature on the ordinary olfactory and trigeminal reflexes has been reviewed in earlier papers. Elsberg, Levy and Brewer have recently confirmed the observations of earlier investigators that many vapors which stimulate the olfactory mucosa also stimulate the trigeminal endings. In 1936 the writer showed that certain vapors which stimulate the olfactory endings in one group of mammals are ineffective in a widely different species of mammal.

Dogs differ somewhat as to the manner in which they first respond to vapors other than the one to which they were first conditioned. Some react at first equally well to new vapors as to the original. Others when given a new vapor if dissimilar to the original, seem confused, become

nervous, turn head in opposite direction, show less fear or anger (less crying or growling), and if the leg responds it is much delayed.

Discrimination between cloves and asafetida was accomplished by establishing a negative conditioned reflex to asafetida after a positive reflex had been established for cloves. The procedure was to shock a delayed response to cloves and never shock for asafetida. Discrimination developed quite rapidly in dogs where the first response to asafetida was much slower than the response to cloves, but when the response was instantaneous for both it was a long and tedious process. Development of a negative trigeminal reflex was not attempted.

There are no grounds for believing that all vapors which are agreeable or disagreeable in one mammal, for example man, are the same for all mammals.

SUMMARY AND CONCLUSIONS

A method is described for eliciting and recording conditioned reflexes from vapors stimulating the olfactory or trigeminal nerves.

It ordinarily takes but 15 to 25 trials to induce the first conditioned response from an "olfactory" or a "trigeminal" vapor. The reflex becomes well-established after a few additional trials.

When limited to the conditions of these experiments, inhalation of cloves, lavender, anise, asafetida, benzol or xylol evokes a conditioned reflex only over the olfactory nerve; while camphor, eucalyptus, pyridin, butyric acid, phenol, sheep dip (sometimes), ether and chloroform are affective over the trigeminal nerve as well as the olfactory.

A negative conditioned reflex can be obtained from a different "olfactory" vapor from the one used for a positive reflex by withholding the shock.

One dog in which the olfactory bulbs were transected and eyes enucleated at birth never acquired an olfactory conditioned reflex or any sensitivity to any of the "olfactory" vapors.

It was demonstrated that practically all vapors which alter respiration over the olfactory or trigeminal nerves are capable of producing a conditioned reflex from stimuli received over the same nerves. Hence a respiratory reflex from any vapor forecasts its conditioned response.

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THE INFLUENCE OF THE PANCREAS ON THE SERUM PHOSPHATASE OF DOGS

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It has been demonstrated that the ingestion of glucose or dextrin temporarily elevates (1) and the ingestion of a high carbohydrate diet persistently elevates (2) the serum phosphatase. According to some observers (3, 4), pancreatectomy augments the serum phosphatase, but a direct relation does not exist between the blood-sugar level and the serum phosphatase activity. Since, as is well known, the serum phosphatase is markedly elevated in certain hepatic and biliary tract disorders in dog and man, and the pancreas is frequently involved in such disorders, we desired to ascertain the effect of that type of pancreatitis and atrophy of the pancreas, which occurs after pancreatic duct obstruction, on serum phosphatase. We also desired to know the effect of diet on the serum phosphatase of depancreatized dogs.

EXPERIMENTAL. *Analytical.* The serum phosphatase was determined by the method of Bodansky (5). Inorganic phosphates were estimated by the Kuttner-Lichtenstein method (6) as modified by Bodansky (7). The same phosphorus method was used for the determination of the organic acid soluble phosphorus of laked whole blood after aliquots of the trichloroacetic acid filtrate of blood had been digested with sulphuric acid. The production of diabetes was verified by blood and urine sugar determinations.

EXPERIMENTAL RESULTS. In table 1A are included the results obtained on two dogs following ligation and inversion of the cut ends of both pancreatic ducts. These results show a slight transient increase in the serum phosphatase with a return to the pre-operative level within a week after duct ligation. Parallel determinations of the organic acid soluble phosphorus of the whole blood showed an increase after ligation of the ducts with a return to the preoperative level in about three weeks. In table 1B are included our findings on 6 dogs, in which the body of the pancreas was removed and the remaining portions separated from the duodenum; this type of operation was used to assure complete exclusion of pancreatic juice from the intestine as well as more complete atrophy of remaining

TABLE 1

The effect of atrophy of the acinar tissue of the pancreas upon the serum phosphatase of dogs

DOG NUMBER	DATE	SERUM		ORGANIC P*	HEMATOCRIT	COMMENTS
		Inorganic P	Pt.			
			Part A			
		mgm.		*mgm.*	*per cent*	
1	6/26/35	5.0	1.30	25.60	60	Diet for 2 weeks before and during experiment consisted of cooked ground meat, 200 grams, raw pancreas and 200 cc. whole milk
	6/27	Pancreatic ducts ligated				
	6/29	5.25	7.35	31.70	49	
	7/5	5.25	1.25	28.10	57	
	7/16	4.78	1.47	27.20	57	Took raw pancreas from diet
	7/19	5.10	1.16	25.85	59	No further change in phosphatase
2	6/26/35	4.30	1.33	22.95	52	Same treatment as dog 1
	6/27	Pancreatic ducts ligated				
	6/29	4.50	7.70	30.30	53	
	7/5	4.75	1.35	24.20		
	7/16	4.00	1.25	22.8	48	Took raw pancreas from diet
	7/19	4.30	1.70	23.3	49	
			Part B			
3	10/6	4.5	0.60			
	10/7	Body of pancreas removed and head and tail separated from gut				
	10/9	4.63	3.57			
	10/18	5.0	4.0	Substituted white bread for the cooked meat		
	10/25	4.10	5.0	Lost appetite, developed diarrhea and vomited		
4	10/13	4.5	2.0			
	10/14	Body of pancreas removed and head and tail separated from gut				
	10/16	4.6	3.0			
	10/25	4.90	2.6	Substituted white bread for the cooked meat in diet		
	11/1	4.15	2.85	Lost appetite; has diarrhea and may vomit after eating No further change in phosphatase		
5	5/27/36	4.60	1.20			
	6/3	Body of pancreas removed and head and tail separated from gut				
	6/6	5.8	2.2			
6	5/27	4.34	1.20			
	6/1	Same operation as dog 5				
	6/6	5.55	3.85			

*This is the total acid-soluble phosphorus of laked whole blood minus the inorganic phosphorus.

TABLE 1—*Concluded*

DOG NUMBER	DATE	SERUM		ORGANIC P*	HEMATOCRIT	COMMENTS
		Inorganic P	Pt.			
			Part B—*Concluded*			
		mgm.		*mgm.*	*per cent*	
7	5/27	5.14	0.76			
	6/3	Same operation as dog 5				
	6/6	6.25	1.5			
8	5/27	4.75	4.5			
	6/1	Same operation as dog 6				
	6/6	4.75	4.3			

acinar tissue. From these results it appears that loss of the external secretion of the pancreas, in addition to atrophy of the pancreas, has little or no influence upon the serum phosphatase value. A high carbohydrate diet for 7 days, or the withdrawal of raw pancreas from the diet (no. 1) likewise had no effect.

Approximately three months after atrophy of the pancreas had been produced the remaining pancreatic tissue was removed from dogs 1 and 4. After these 2 dogs had been diabetic for some time their common bile ducts were ligated. Table 2A includes the results obtained upon these dogs; this table shows that pancreatectomy caused some elevation of the serum phosphatase which tended to persist for a considerable length of time. It also shows that large quantities (100 gm./day) of cane sugar has no significant influence upon the serum phosphatase of the diabetic dog. Dog 1 showed an increased serum phosphatase after being on a high carbohydrate diet for 5 days while similar treatment of dog 4 was without effect on the serum phosphatase. This question was not followed further because of the inability of the dogs to tolerate the type of diet used for these experiments. One of these dogs (no. 1) was deprived of raw pancreas for 27 days without a significant effect upon the serum phosphatase; the other dog (no. 4) was maintained without insulin for 21 days without any note-worthy change in the serum phosphatase. Common bile duct ligation caused the marked elevation of serum phosphatase which is known to occur (8) in non-diabetic dogs. The pre-operative diet of these dogs had contained no sugar as such. Shelling (4) used Cowgill's diet (9) (containing 34.9 per cent cane sugar) and found a pre-operative level of 1 to 2 units, with an elevation of the serum phosphatase after pancreatectomy of from 20 to 30 units.

We thought that the magnitude of elevation of serum phosphatase might be influenced by the sugar content of the diet prior to pancreatec-

TABLE 2

The effect of removal of the pancreas upon the serum phosphatase of dogs

Some of the dogs reported in this part were used in the preceding series and when such is the case the dog's number will indicate this fact.

DOG NUMBER	DATE	SERUM VALUES		REMARKS
		Inorganic P	Pt.	
		Part A		
1	9/29	4.20	1.30	
	10/4	Depancreatized.		Hard fibrous pancreas
	10/6	5.15	5.85	20 clinical units of insulin daily. Diet is pancreas, meat, milk and some cereal
	10/13	5.60	5.0	Fasting blood sugar 12 hrs. after insulin 380 mgm./100 cc.
	10/15	Started this dog on a high carbohydrate diet consisting of Karo, white bread, raw pancreas and milk		
	10/16	Dog eats, but spirits depressed		
	10/17	Vomits food—diarrhea		
	10/18	Refused to eat white bread. This dog has always had a voracious appetite		
	10/18	7.48	12.1	Returned to meat diet
	10/25	6.5	4.4	Fasting blood sugar 415 mgm./100 cc.
	11/1	5.8	3.4	
	11/8	Same diet minus raw pancreas		
	11/14	4.90	5.60	
	11/29	4.20	5.80	Fasting blood sugar 490 mgm./100 cc.
	12/3	Returned pancreas to diet and also 100 grams of cane sugar added to diet daily		
	12/6	4.0	7.0	
	12/13	4.80	7.0	Left off cane sugar
	12/19	5.35	7.0	Ligated common bile duct Duct larger than normal
	12/20	4.50	7.80	
	12/23	5.44	46	Icteric index 75. Van den B+
	12/29	4.15	60	Last insulin on 25th
	1/3/36	6.34	57.6	Blood sugar 30th. 644 mgm./100 cc.
4	11/1	4.15	2.85	20 units of insulin daily
	11/4	Head and tail of pancreas removed		
	11/8	4.0	7.0	Fasting blood sugar 200 mgm./100 cc. Place on a high carbohydrate diet, pancreas and milk
	11/14	4.70	7.5	Stopped insulin
	11/29*	3.20	5.90	Fasting blood sugar 21st. 510 mgm.
	11/30*	Added 100 gm. of cane sugar to diet. Still no insulin		
	12/5*	3.45	5.0	Started insulin. Blood sugar 415 mgm./100 cc.
	12/13	3.60	3.23	

* No insulin.

TABLE 2—*Concluded*

DOG NUMBER	DATE	SERUM VALUES		REMARKS
		Inorganic P	Pt.	
		Part A—*Concluded*		
4	12/19	3.84	5.60	Ligated common bile duct, which appeared larger than normal
	12/20	4.10	5.90	
	12/23	4.50	38.50	Eats very little. Van den B positive
	12/27	4.35	48.15	
	12/30	Killed.		Bile peritonitis due to ruptured duct
X	8/5	4.55	1.40	Before pancreatectomy
	8/8	3.00	6.85	
	8/10	5.50	1.60	
		Part B		
9	5/28	3.40	2.3	Just before pancreatectomy. Standard diet: meat, cereal, raw pancreas, and milk, also cane sugar
	5/29	5.0	7.4	15 clinical units of insulin daily
	5/30	5.0	9.2	
	5/31	4.7	10.3	
	6/1	4.9	15.3	
	6/3	5.1	14.3	
	6/7	4.6	9.0	
	6/14	4.4	6.1	
10	5/28	3.0	2.0	Just before pancreatectomy. Same diet as dog 9
	5/29	5.1	5.9	15 clinical units of insulin daily
	5/30	5.10	8.8	
	5/31	5.15	9.35	
	6/1	4.75	8.95	
	6/3	5.25	8.75	
	6/7	5.4	8.4	
	6/14	5.17	4.83	
11	6/8	4.16	3.19	Just before pancreatectomy. Same diet as dog 9
	6/9	4.66	12.34	15 clinical units of insulin daily
	6/11	4.40	18.60	
	6/13	4.55	17.15	
12	6/8	4.35	1.65	Just before pancreatectomy. Same diet as dog 9
	6/9	5.35	2.15	15 clinical units of insulin daily
	6/11	6.0	5.95	
	6/13	5.9	5.3	

tomy. To test this point 4 dogs were maintained for 1 month on the diet previously used plus 100 grams daily of cane sugar. Table 2B shows the results obtained on these 4 dogs. Three of the 4 showed a serum phosphatase elevation which was considerably greater than that recorded in table 2B after pancreatectomy.

To see whether or not pancreatic juice, like bile, contains phosphatase we determined the phosphatase content of the former. The secretion was collected in acute experiments after secretin injection or from dogs with chronic pancreatic fistula. The results are recorded in table 3 and show that pancreatic juice of the dog contains appreciable phosphatase activity. The concentration, however, is considerably less than that found in bile as reported by Armstrong and co-workers (14) (these workers used a different substrate for enzyme estimation), or by ourselves, in which we used the same method on both secretions. Our values for dog's bile, obtained from chronic fistula, ranged from 118 to 15 units per 100 cc. of

TABLE 3

Phosphatase content of pancreatic juice

DOG NUMBER	PHOSPHATASE	REMARKS
	units/100 cc.	
1	11.0	Secretion stimulated
2	11.6	Secretion stimulated
3	9.6	First day—chronic drainage
	9.3	Second day—chronic drainage
	9.0	Third day—chronic drainage
4	9.0	Chronic drainage

bile; the low values were obtained after the bile had become infected. (It was found necessary in the case of both bile and pancreatic juice to add acid-washed activated charcoal to the incubated acidified mixture before filtration, in order to obtain a water-clear filtrate which would give no turbidity with the phosphorus reagents.)

We found, in two experiments, that ether anesthesia *per se*, or freeing adhesions from the liver, as used in our operations, did not influence the serum phosphatase beyond the range of normal variation.

Discussion. The serum phosphatase is known to be elevated in certain bone diseases (10) and in obstructive jaundice (11). However, the significance or origin of serum phosphatase is not understood. Armstrong and Banting (12) believe that bone is the sole source of serum phosphatase while Bodansky suggests that its increase during carbohydrate absorption may be a part of the absorption process or of the metabolism of carbohydrates. Phosphorylation in an extract of intestinal mucosa is in-

hibited by iodoacetate, according to Wilbrandt and Laszt (16). Lundsgaard (13) found that phloridzin decreases the phosphatase activity of muscle and also the production of lactic acid from glucose or glycogen. He suggests, but was unable to prove, that the poisoning of kidney phosphatase by phloridzin may be the reason for a failure of glucose resorption by the tubules. It is not clear whether such sources as kidney or intestinal phosphatase contribute to the concentration of this enzyme in the serum, although the removal of such organs does not decrease the serum phosphatase, according to Armstrong and Banting (12).

Irrespective of its significance, one must include an elevation of the serum phosphatase as a change occurring in the blood following pancreatectomy in the dog. This change occurs whether a normal or a fibrotic pancreas is removed; it occurs irrespective of insulin and raw pancreas therapy. The feeding of a diet rich in cane sugar prior to pancreatectomy augments the serum phosphatase elevation following this operation, while instituting the same diet after removal of the pancreas fails to influence the activity of this enzyme in the serum.

The degenerative changes which occur in the pancreas after occlusion of its ducts results in only a slight temporary rise in serum phosphatase which is in contrast with the more marked and prolonged elevation which occurs after obstruction of the common bile duct. Occlusion of the common bile duct in the depancreatized animal produces the typical rise in serum phosphatase which rules out the pancreas as a significant factor in the genesis of the rise and has definite implications in the differential diagnosis of diseases of the pancreatic and hepatic structures.

Although phosphatase is present in pancreatic juice, its presence there can hardly afford a plausible explanation of the serum rise after duct ligation, since pancreatectomy causes a more decided elevation; and, by analogy, these facts lead one to wonder whether or not the damming back of bile is an adequate explanation of the serum phosphatase elevation which occurs in common duct obstruction. It may be that the rise in serum phosphatase after ligation of the pancreatic ducts or removal of the pancreas is due to the hepatic changes. The greatest changes in the liver occur after pancreatectomy (15). However, the change in phosphatase occurs with raw pancreas (200 grams daily) in the diet in amounts which prevent the fatty changes in the liver of the depancreatized dog receiving insulin; while the removal of raw pancreas from the diet of the dogs with pancreatic ducts ligated and from one depancreatized dog did not affect the serum phosphatase. The effect of a high carbohydrate diet upon the serum phosphatase (1, 2) inclines one to believe that the changes following pancreatic removal or degeneration of acinar tissue are in some way related to a disturbance in carbohydrate metabolism.

SUMMARY AND CONCLUSIONS

Pancreatic juice, like bile, contains phosphatase, but atrophy of the pancreas, produced by ligation of the ducts or separation of the pancreas from the duodenum, has but slight effect upon the serum phosphatase activity. However, removal of the pancreas invariably results in an increase in serum phosphatase activity which may vary from a few units to several times the initial level, but the rise is not as high as following occlusion of the common bile duct. The elevation after pancreatectomy is followed by a decline of the serum phosphatase.

Absence of the pancreas does not inhibit the additional rise in serum phosphatase which occurs after ligation of the common bile duct. The cane-sugar content of the pre-operative diet influences the magnitude of the serum phosphatase elevation following pancreatectomy. The presence or absence of raw pancreas in the diet does not significantly alter the serum phosphatase.

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THE INFLUENCE OF NUTRITIONALLY PRODUCED ANEMIA ON THE ABILITY OF EXPERIMENTAL ANIMALS TO WITHSTAND LOW TEMPERATURES

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Energy and heat production are both dependent upon oxidative changes occurring in the tissues. A full satisfaction of the oxygen demands of the tissues is, in turn, dependent upon the ability of the blood to transport oxygen in adequate amounts. The number of erythrocytes and the amount of hemoglobin also condition this latter response. Since there is a constant breakdown of red cells and hemoglobin, there must be a corresponding production of these same materials. This necessitates a continual supply of those essentials necessary for the fabrication of these elements and for the factors which are required by the bone marrow in the maturation and delivery processes. Under experimental conditions the supply of these materials can be withheld partially or entirely by properly adjusting the food supply.

In the experiments herein described yeast constituted the only variable in the diet. In a former report (1) the value of yeast in providing the materials essential for erythrogenesis was determined by utilizing, as a method for stimulating erythropoiesis, exposures to rarefied atmospheres. This report will deal with the influence of anemia, nutritionally produced, on the ability of the experimental animal to withstand low temperatures. The impetus for this study grew out of a series of observations made by the senior author while he was compiling data for his Ph.D. thesis (2). It was observed at that time that many of the young born of mothers receiving an insufficient amount of vitamin B showed a severe anemia. "Upon going into the laboratory in the morning, it was a frequent experience to find individuals or entire litters dead. Careful autopsies gave no clue as to the cause of death. The marked pallor of the animals made it appear that death might in some way be related to the anemic condition." The mornings that such observations were made were always preceded by cold nights when the temperature in the laboratory fell to an unusually low level in spite of the fact that it was located immediately over the boiler room. One of us (I. A. M. (15)) has already described a vitamin B deficiency—beriberi—developing for the first time in the second genera-

tion. It was shown at that time that the food material carrying the vitamin B complex had to be increased nearly 400 per cent in the mother's diet before her young would be protected from the development of polyneuritis.

Polyneuritis was not found to be a complicating factor in the anemic young who, so many times, were incapable of surviving sudden extreme drops in temperature. At least the polyneuritis was not severe enough to manifest itself in paralysis. It is realized, of course, that the young individual is not as capable of adjusting his temperature to as marked changes as the adult. While this may be a factor in the observations just noted, it is not believed to be the sole condition responsible for the findings. The reason for this is that most of the animals dying under these peculiar circumstances had lived well beyond the weaning age. Furthermore, in the experiments to be described, the animals were young adults several months old.

METHOD. Young white rats were separated from their mothers as soon as weaned and placed upon a basal diet of the following composition: Casein (Harris) 18; cod-liver oil (Mead) 3; Crisco 2; salt mixture (3) 4; and bleached dextrin 73. The animals were arranged in groups of four and fed the basal diet alone or supplemented with various amounts of yeast (Harris Brewer's). They were maintained on this regime until there was produced a definite anemia and a drop in rectal temperature. It was noted early in the experiment that all yeast supplements above one per cent enabled the animals to maintain normal blood values in respect to red cells and hemoglobin. Anemia developed only when the diet was nearly or entirely devoid of yeast. That there was very little if any storage of yeast factors is exemplified by the rapid development of anemia once the yeast was removed. This confirms recent findings of others (4).

As soon as there was definite evidence of anemia, an animal was placed in a small open cage which, in turn, was placed in an electric refrigerator. The refrigerator was equipped with a thermostatic control which permitted the choice of seven different temperatures, ranging from 3°C. at position seven to −7°C. at position two. At position one, the cooling apparatus ran continuously with the result that quite low temperatures were capable of being attained. The thermostatic control was so efficient in operation that it maintained a constant temperature when the control button was set in a given position. The refrigerator was equipped with a glass door, so that observations could be made of the temperature and of the conditions of the animals.[1] A centigrade thermometer was suspended

[1] The authors wish to take this opportunity to thank the Westinghouse Company and their local representative, Mr. Russell K. Akin, for their most cordial coöperation in preparing the equipment for these experiments.

just inside the door in such a manner that readings from the outside could be made. A four hour period of exposure was arbitrarily decided upon.

RESULTS. *Rapidity of onset of anemia.* On September 6, 1934, when the animals of group I were placed on the basal diet, their weights averaged 51 grams. By September 27, the average red cell count was 7.531 million and the hemoglobin 123 per cent. The weight averaged 68 grams. On October 17, the red cell count had fallen to 5.97 million, the hemoglobin to 91 per cent and the average weight to 56 grams. Thus, in twenty days, the blood changed from normal values to anemic ones. An examination of the record of group II (table 1) not only shows that an anemia is capable of developing on the basal diet containing one per cent yeast, but that a period of fifteen days is required. The animals of group III were definitely anemic on December 27, 64 days after the yeast had been reduced to one per cent. The animals in group IV showed a marked drop in hemoglobin, red cells and body temperature on January 3, three days after all yeast had been removed from the diet. It should be borne in mind, however, that the period of total deprivation of yeast followed a period of 68 days when the food contained only one per cent yeast. The anemia developed by the animals in group IV was not, therefore, entirely due to the complete absence of yeast. The animals in group V also showed an anemia when the amount of yeast was reduced to one per cent or omitted entirely. The shortest period of time (15 days) in which an anemia developed with a low inclusion of yeast is shown by the animals in group II.

In a previous report (1) it was shown that a deficiency of the B-complex results in a terminal anhydremia with a resultant increase in red cells and hemoglobin. In the instances cited above the deprivation of the B-complex was either not severe enough or had not extended over a sufficient period of time to produce anhydremia. The evidence substantiating this statement is based on determinations of the specific gravity of blood. The specific gravity of the blood of a normal rat was found to be 1.065. The specific gravity of the blood of five anemic rats averaged 1.0652.

Order of appearance of signs of deficiency. On December 6, rats 5, 7 and 8 of group II showed a definite loss in weight. Six days later (December 12) the body temperature was still normal. Rats 10, 11 and 12 of group III suffered a distinct loss in weight during the period between December 6 and December 12. At this time there was no drop in body temperature and as late as the 27th, the body temperature had dropped only slightly, as will be seen in table 1. Rats 13, 14, 15 and 16 of group IV experienced the first marked decline in weight during the period between December 11 and 21. At this time their body temperatures were normal and were still so on December 26. On January 3 the temperatures of rats 14 and 16 showed a definite decline. Rats 17, 18, 19 and 20 of group V showed their first definite loss in weight on December 21. On December 26 their tem-

TABLE 1

DATE	RAT NUMBER	R.T.	RBC	Hb
		Group I		
				per cent
9/ 6/34	Started on basal diet only			
9/27/34	#3		7.600	123
10/17/34	#2		5.970	84
	#3		5.820	92
	#4		6.120	96
		Group II		
8/22/34	Started on basal diet supplemented with 2% yeast			
10/ 1/34	#6		8.450	122
10/16/34	#6		6.960	104
10/24/34	Changed to diet with only 1% yeast supplement			
11/ 8/34	#6	98.3	7.000	86
12/26/34	#5	98.0	6.900	88
	#7	99.2	6.840	92
	#8	99.0	6.900	90
		Group III		
8/22/34	Started on diet supplemented with 4% yeast			
10/ 1/34	#11		6.587	127
10/24/34	Changed to diet with only 1% yeast supplement			
10/24/34	#11		7.940	124
12/27/34	#10	100.9	7.000	96
	#11	101.4	7.200	94
	#12	101.3	7.440	98
		Group IV		
9/ 1/34	Started on diet supplemented by 8% yeast			
9/27/34	#14		6.275	111
10/24/34	Changed to diet with only 1% yeast supplement			
10/24/34	#14		7.880	118
1/ 1/35	Changed to diet without any yeast supplement			
1/ 3/35	#14	99.2	6.992	95
	#16	99.4	6.872	98
1/10/35	#13	98.6	6.112	94
	#15	100.6	6.424	92
		Group V		
9/ 6/34	Started on diet supplemented by 12% yeast			
9/26/34	#17		7.200	106
10/24/34	Changed to diet with only 1% yeast supplement			
10/24/34	#17		7.780	114
1/ 1/35	Changed to diet without any yeast supplement			
1/10/35	#19	99.6	6.840	101
2/11/35	#17	94.0	7.000	95
	#18	95.2	6.970	98
	#19	95.1	6.840	99

peratures were still normal. On January 10 the temperatures of rats 17 and 19 were definitely lowered. Thus it will be seen that loss of body weight occurs not sooner than five days prior to declines in body temperature. The probability is that there is a still greater interval between these two signs of vitamin deficiency.

Minimal amount of yeast required to prevent anemia. The animals in group II received the two per cent yeast supplement from the 22nd of August to the 24th of October. Red cell counts and hemoglobin determinations done on October 1 and 16 showed very little, if any, evidence of a developing anemia. This covered a period of 55 days. On October 24 the yeast was reduced to one per cent. November 8, rat 6 had seven million red cells, 86 per cent hemoglobin and a body temperature of 98.3°F. Thus in 15 days there was definite evidence of anemia. Group III were started on the four per cent yeast supplement August 22. On October 24, the yeast was reduced to 1 per cent after a second red cell count and hemoglobin determination showed that there was no evidence of anemia. Thus over a period of sixty-three days, the 4 per cent yeast supplement was sufficient to maintain normal blood levels. On December 27, 64 days later, blood determinations showed the presence of a mild anemia. It may be concluded from this evidence that one per cent yeast is not sufficient to prevent the development of an anemia, and that an inclusion of 4 per cent yeast that is later reduced to 1 per cent delays the appearance of the anemia because of a storage or carry-over effect.

Relation of drop in body temperature to decreases in the amount of hemoglobin and the number of red corpuscles. A survey of the records does not reveal a single instance in which the body temperature suffered a decline without a corresponding diminution in the per cent of hemoglobin and the number of erythrocytes. A survey of the records of the fifteen animals in whom both body temperature and per cent hemoglobin were determined when anemia prevailed will show that there is a tendency for the decline in body temperature to parallel the drop in the percentage of hemoglobin. There was also a corresponding decrease in the number of red cells. Up until the onset of anhydremia, which should be considered as a terminal affair in a deficiency of the B-complex, there appears, therefore, a coincident drop in body heat, red corpuscles and hemoglobin.

Responses of anemic and normal rats to low temperatures. On September 27, 1934, the animals in group I had an averaged red cell count of 7.531 million and an average of 123 per cent hemoglobin. Reference to table 2 will show that these animals were capable of withstanding a temperature of −4.0°C. for 21.5 hours. At this point we wish to call attention to the fact that while this group of experimental animals were in the refrigerator for 21.5 hours, they showed no signs indicative of a shortage of oxygen. It might be thought that a tight compartment of 7.5 cubic feet capacity

TABLE 2

DATE	TIME	RAT NUMBER	TEMPERATURE		RESULTS
			C.	F.	
Group I					
9/ 6/34	Started on basal diet only				
10/ 9/34 to 10/10/34	11:30 a.m. to 8:00 a.m.	#1, 2, 3, 4	−4.0	24.8	Survived
10/18/34	8:15 a.m.	#2, 3, 4	4.5 to 3.0	40.1 to 37.4	Died in 4 hours
Group II					
8/22/34	Started on diet supplemented by 2% yeast				
10/22/34	1:15 p.m.	#5, 6, 7, 8	0 to −0.25	32.0 to 31.55	Survived
10/24/34	Changed to diet with only 1% yeast				
11/ 9/34	8:30 a.m.	#6	7.25	45.05	Died in 4 hrs.
12/26/34	11:00 a.m.	#5, 7, 8	6.5	43.7	#5 died in 2½ hrs. #7, 8 surv.
Group III					
8/22/34	Started on diet supplemented by 4% yeast				
10/24/34	Changed to diet with only 1% yeast				
12/31/34	1:00 p.m.	#10, 11, 12	1.0	33.8	2 survived, 1 died in 3½ hrs.
1/ 1/35	Diet changed to omit the yeast entirely				
1/ 2/35		#11, 12	−3.0	26.6	Survived
Group IV					
9/ 1/34	Started on diet with 8% yeast supplement				
10/24/34	Changed to diet with 1% yeast supplement				
1/ 1/35	Diet changed to omit yeast entirely				
1/ 4/35	1:00 p.m.	#14, 16	5.5	41.9	Survived
1/ 5/35	9:00 a.m.	#14, 16	2.5	36.5	1 died in 1 hr. 40 min.; 1 died in 1 hr. 25 min.
1/13/35	1:00 p.m.	#13, 15	5.5 to 6.0	41.9 to 42.8	#13 died in 4 hrs. #15 surv.
1/14/35	9:00 a.m.	#15	2.0	36.2	#15 died in 1 hr.
Group V					
9/ 6/34	Started on diet with 12% yeast supplement				
10/24/34	Changed to diet with 1% yeast supplement				
1/ 1/35	Diet changed to omit yeast entirely				
1/17/35		#19	−4.0	24.8	Survived
2/11/35		#17, 18, 19	6.0	42.8	#17 died in 1 hr. #18, 19 died in 3 hrs.

would not contain enough oxygen for normal requirements or for requirements slightly in excess of normal. Obviously, there is either enough oxygen inclosed or enough circulation to maintain an adequate oxygen tension. On October 18, when nutritional anemia was definite, the animals were again placed in the refrigerator. The initial temperature was 4.5°C. and the final temperature at the end of four hours, 3°C. The animals were not able to survive this test. The anemia was of such a degree that life was not possible at a temperature of 37.4°F.

On October 16 the animals in group II averaged 7.325 million red cells and 107 per cent hemoglobin. The following week these animals were exposed to temperatures as low as −0.25°C. without suffering any ill consequences. On October 24 the yeast was reduced to one per cent. On November 8 rat 6 with a red cell count of 7.000 million and a hemoglobin of 86 per cent was placed in the refrigerator with the temperature set at 7.25°C. It was not able to survive the four hour exposure. This animal therefore succumbed to a temperature of 45°F. On December 26, the remaining animals of this group averaged 6.88 million red cells and 90 per cent hemoglobin. When placed in the refrigerator at a temperature of 6.5°C. one died in 2.5 hours and two survived. Here it will be seen that the animals with a hemoglobin of 88 per cent or less and a body temperature of 98.3 or less were unable to survive a temperature of 43.7°F. while animals with a hemoglobin of 90 per cent or more and a body temperature of 99.0°F. or more were capable of withstanding this temperature.

The animals in group III did not show any evidence of anemia until after the yeast supplement had been reduced to one per cent. Sixty-five days later only a slight anemia prevailed—the hemoglobin being not less than 94 per cent, the red cells not less than seven million and the body temperature not less than 100.9°F. With blood values such as these, two animals were capable of withstanding a temperature of −3°C. while one died after 3.5 hours' exposure to a temperature of 1°C.

On January 3, 1935, rats 14 and 16 of group IV showed a mild anemia. They survived four-hour exposures to temperatures of 6.5°C., 6.0°C. and 5.5°C. When the temperature was reduced to 2.5°C. one died in one hour and 25 minutes and the other in one hour and 40 minutes. One week later, the remaining two animals of this group (rats 13 and 15) with a more severe anemia, were not able to survive a temperature of 2°C. This is in marked contrast to the animals in group III, who, with a higher amount of hemoglobin, red cells and body heat, were able to withstand the effects of a temperature of 2°C. for four hours.

On January 10, 1935, rat 19 of group V had a red cell count of 6.84 million, 101 per cent hemoglobin and a body temperature of 99.6°F. This animal successfully withstood four hours' exposure to a temperature as low as −4°C. A month later, however, this animal, together with

the others of this group, could not survive a temperature of 6°C. (42.8°F.). A review of these results will show that rats are capable of withstanding temperatures as low as 24.8°F. if the number of red cells, the amount of hemoglobin and body temperature are high enough. On the other hand, when these figures are low, temperatures no lower than 45°F. prove to be lethal.

The effect of increasing amounts of yeast on the hemoglobin and erythrocytes. An examination of table 3 will show that the basal diet alone is productive of an anemia marked by a diminution in both red cells and hemoglobin. Groups II and III, receiving a supplement of 2 and 4 per cent yeast, experienced a slight drop in hemoglobin, but a rise in red cells. Groups IV and V, receiving 8 and 12 per cent yeast, were capable of increasing both red cells and hemoglobin. When yeast is present in the diet to the extent of 8 per cent or more, the animals are able to increase both red cells and hemoglobin. When yeast is present only to the extent of 2 to 4 per cent, the red cells only are capable of being increased, while the hemoglobin

TABLE 3

GROUP NUMBER	PER CENT YEAST SUPPLEMENT	FIRST DETERMINATION		SECOND DETERMINATION	
		RBC	Hb	RBC	Hb
			per cent		*per cent*
I	0	7.531	123	5.970	91
II	2	6.882	114	7.325	107
III	4	6.915	120	7.657	117
IV	8	6.370	108.5	8.080	121
V	12	7.321	102	7.508	108

suffers a decline. Evidently a restriction in yeast is first manifested by a curtailment in hemoglobin production, later by a reduction in red cell output. When the diet contains only one per cent or less yeast, both elements decrease. Here is confirmation of evidence already presented (1) that a fluctuation in hemoglobin is a more delicate index of the amount of B-complex in the diet than that provided by changes in the number of red blood corpuscles.

Discussion. This laboratory, as well as others (5, 6, 7, 8), has shown that dogs suffering from a deficiency of the B-complex are incapable of normal gastric secretory and motor activity. The same effect may be presumed to be true for rats (9). The existence of a partial or complete absence of hydrochloric acid from the stomach seriously impairs the absorption of iron. A co-existing anorexia reduces the amount of food eaten. Both factors, therefore, act to reduce not only the intake of essential blood building materials but seriously impair their digestion and absorption as well. Pregnancy also is known to be accompanied frequently

by a reduction in the secretion of hydrochloric acid. This condition is assumed to play a major rôle in the development of anemia due to the impairment of iron absorption. It is doubtful, however, that the full benefit of the B complex is due to the ability of vitamin B_1 to maintain normal gastric secretory and motor activity. Alvarez and his co-workers (4) noted the development of an anemia which appeared as the result of a restriction of the vitamin B_1 intake. Since there appeared to be no reduction in the amount of hydrochloric acid produced by these people, it does not seem likely that there was any impairment in iron absorption. These workers also noted that the loss of hemoglobin was more marked than the loss of erythrocytes.

Sure (10) has stated that vitamin B deficiency produces anhydremia. We have obtained similar results but found that the anhydremia was a terminal affair. In the experiments herein described, care was taken to choose animals before any anhydremia had developed. This report calls attention to the drop in body temperature which is associated with other signs of a vitamin B deficiency. Undoubtedly these animals suffer from a cold environment much more than animals with the ability to maintain a normal body temperature. Barbour (11) states that "the degree of blood hydration runs parallel with body temperature. . . . Fever and antipyresis exhibit respectively the reactions induced by cold or hot environments." The blood is concentrated by cold and diluted by heat. Since, in vitamin B deficiency the body temperature drops due either to a coexistent anemia or a reduction in the oxidative processes in the tissue cells or both, it is not unreasonable to assume that the terminal anhydremia with the corresponding rise in hemoglobin and red cells simply represents an attempt on the part of the body to compensate for what appears to it to be a cold environment.

The tissues of the organism can suffer from an oxygen deprivation because of the following conditions: reductions in barometric pressure or oxygen tension of the surrounding atmosphere, impairment of the aerating surface of the lungs due either to a reduction in surface area or in the permeability of the pulmonary epithelium and lastly to defects in the circulation due to either organic or functional disturbances of the heart. Admittedly impoverishment of the blood either in respect to a reduction in the number of red cells or in the amount of hemoglobin or both, will also result in a reduction of oxygen delivery to the tissues. In this discussion, however, we wish to picture the blood as a normal medium which is capable of bringing about compensatory adjustments to meet the deficiencies just mentioned. If atmospheric conditions and the status of the heart and lungs are known to be within normal limits, then any variation in the ability of the blood to transport oxygen to the tissues in adequate amounts will be made manifest by various evidences

of reduced heat and energy production. On the other hand, if an experimental animal under such conditions seems to effect any degree of compensation, the mechanism of this must be sought for in the tissues supplied by the peripheral circulation.

Clinically, patients suffering from pernicious anemia have had their hemoglobin reduced to as low as 21 per cent and their red cells to 1.184 million without suffering fatal consequences. They suffer acutely from low environmental temperatures, so that the question naturally arises as to whether or not they would succumb as did the animals described in this report from exposure to temperatures of 45°F. When a patient with severe pernicious anemia dies, and death cannot be ascribed to some intercurrent cause, to what may death be due? What are the outstanding pathological findings? For the purposes of this discussion, the chief changes found at autopsy of persons dying of pernicious anemia are, aside from fatty degeneration, pallor and pigment deposits, the dark red color of the muscles and the extensive occurrence of hemorrhages. Kaufmann (12) says, "Hemorrhages in various organs can almost always be found and are most remarkable in the serous membranes, especially the pericardium beneath the endocardium, in the meninges, background of the eye, brain and spinal cord which may also show foci of degeneration."

Pathological findings in death caused by freezing bear an interesting similarity to those described above. Bender (13) described miliary hemorrhages in all parts of the brain along with pulmonary edema and hemorrhages. Bender concludes her description by saying, "the miliary hemorrhages were identical in all ways with those seen in the brains of human beings and guinea pigs as to their distribution, size, relation to the capillaries and absence of cellular or tissue reaction; such hemorrhages do not form a part of the pathologic picture of encephalitis."

The pathological changes occurring in pernicious anemia and in freezing are as remarkable in their paucity as they are in the frequency of hemorrhages. Death in either instance is a matter of a few hours. Freezing, however, constitutes an acute condition while pernicious anemia is one lasting over several months or years. The color changes in the latter condition are due to blood pigment alterations but death, in either instance, is due to a final inability to maintain a proper degree of body temperature. Barbour (11) states that life is not possible when the body temperature exceeds certain limits (26° to 42°C.). In the experiments herein described an instance was cited of death occurring at an environmental temperature of 45°F. when the red cells were reduced to seven million and the hemoglobin to 86 per cent. It does not seem probable that a human being suffering with pernicious anemia would suffer such an outcome even if the temperature were lower or if the red cell count and per cent of hemoglobin were less. While there is good reason to believe that

the cause of death in both of the instances cited above is due to a drop in body temperature below that compatible with life, it is difficult to understand why, in pernicious anemia, death should occur with a hemoglobin around the level of twenty per cent and the number of red cells as low as one million, and why, in a nutritionally produced anemia, death from low temperatures occurs with a hemoglobin not lower than eighty per cent and the red cell count around six million. The explanation may lie in the compensatory mechanisms which the patient with pernicious anemia may be able to effect because of the chronic nature of his disease and which the experimental animal cannot bring about because of the acute nature of the experiment. It seems to the authors that the chief compensation which the pernicious anemia patient brings about in order to maintain a body temperature within normal limits is the remarkable production of muscle hemoglobin—the pigment producing the dark red color so characteristic of the musculature of these patients. Muscle hemoglobin is entirely distinct from blood hemoglobin and by its presence enables muscle tissue to abstract oxygen from the circulating blood under greatly reduced tensions. In this way the presence of muscle hemoglobin would guarantee a continuation of normal oxidative processes in the cells beyond the limits usually considered possible. For example, it would be difficult or impossible for a normal individual to survive the experimental reduction of his hemoglobin to twenty per cent and his red cells to one million. Woodruff and Whipple (14) have reported two muscle hemoglobin determinations in humans dying of aplastic anemia. One had a value of 1,000 mgm. per 100 grams of muscle and the other had 1,090 mgm. The hemoglobin was 43 and 30 per cent respectively and the red cell count in millions 2.6 and 1.74. In none of the other cases in which the hemoglobin and red cells were reported were the values as low. If, in the cases cited by Woodruff and Whipple, the muscle hemoglobin is plotted against blood hemoglobin, it will be found that as the blood hemoglobin rises the muscle hemoglobin falls. Pneumonia proved to be an exception to this rule. In pneumonia there is oxygen deprivation because of an impairment or reduction in the pulmonary epithelium. The hematopoietic and muscle tissues being normal, an attempted compensation is sought by increases in both muscle and blood hemoglobin. Pernicious anemia is not characterized by impaired pigment production. The color index is high and the amount of muscle hemoglobin is markedly increased. This is not true for vitamin B deficiency since there is a marked decrease in blood pigment as well as the number of red cells.

There are three variables which are thus concerned in the delivery of oxygen to the tissues, namely: muscle hemoglobin, blood hemoglobin and the red blood corpuscles. If changes in barometric pressure, oxygen tension, cardiac or pulmonary defects threaten to bring about an oxygen

deficiency, all three of these mechanisms are brought into activity to effect an adequate compensation. It might seem, therefore, that the product of these three values might represent a physiological constant below which life is not possible, and that part of the functions of the vitamin B content of yeast is to enable an animal to effect these changes.

CONCLUSIONS

1. White rats when placed on a purified ration containing one per cent or less of brewer's yeast as the sole source of the B-complex develop an anemia in from two to three weeks. A diet containing four per cent yeast which is later changed to one per cent delays the appearance of anemia due probably to a storage or carry-over effect.

2. The loss in body weight resulting from a deficiency of the B-complex definitely precedes a lowering of body temperature which is also characteristic of this deficiency.

3. Prior to the onset of anhydremia and following the onset of body weight losses, there is a coincident drop in body heat, the number of red blood corpuscles and the amount of hemoglobin.

4. The anhydremia developing late in vitamin B deficiency is explained as being due to a shift in water from the blood to the tissues which constitutes a natural readjustment of an animal exposed to a cold environment, or to an animal who feels cold because of an impoverishment of the blood.

5. The ability of animals to withstand low temperatures progressively decreased as the severity of the anemia increased.

6. A gradual reduction in the amount of yeast in a diet otherwise devoid of the vitamins contained in this material is first marked by a reduction in body weight, then hemoglobin and later by a reduction in the number of red cells.

7. It is not believed that the full benefit of the B-complex in maintaining normal blood values is due solely to its ability to promote normal appetite, digestion and absorption.

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STUDIES OF THE URINE AT HOURLY INTERVALS AFTER THE ADMINISTRATION OF GLYCINE

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During investigations of the effects of the prolonged administration of glycine on the daily excretion of various substances in the urine, creatinine and creatine in particular (1), it seemed of interest and importance to include also a series of observations in which the more immediate changes in composition of the urine were determined within the first few hours after the ingestion of a single large dose (10 to 20 grams) of glycine.

EXPERIMENTAL PROCEDURES. The individuals who consented to serve as the subjects of these observations were allowed to take their customary mixed diets, but on the morning of an experiment they came to the laboratory without breakfast. Each subject remained in the laboratory throughout the period of observation, for the most part quietly sitting in a chair. After the urine had been collected for a preliminary period of two hours, the subject was given 10 to 20 grams of glycine dissolved in 200 cc. of water, or $\frac{3}{8}$ grain (0.024 gram) of ephedrine sulfate in a similar amount of water, or both glycine and ephedrine in water, or water alone. Specimens of urine were then collected at intervals of one to two hours for a period of four to six hours. In order to have a sufficiently large volume of urine the subject was given 200 cc. of water at the beginning of each of these intervals. The values for the nitrogen and sulphur partition products were determined in each specimen of urine and in some instances the values for inorganic phosphate, sodium, potassium, calcium and magnesium were also determined. Samples of blood usually were obtained at intervals corresponding to the collections of urine. The blood was analyzed for urea, amino acids, uric acid, creatinine, and creatine. The analytical methods used have been described in a previous paper (2).

The subjects observed in this manner included four normal individuals (fifteen experiments), eight patients who had myasthenia gravis (fourteen experiments), five individuals who had progressive muscular dystrophy (fifteen experiments), and five subjects who were suffering from chronic exhaustion, easy fatigability, or similar conditions (eight experiments).

RESULTS. There are relatively few data in the literature of the type obtained in these observations; consequently, we have selected typical

experiments for presentation in some detail. The results of a series of experiments in which the subject was a normal young woman are summarized in table 1. In two control experiments in which water alone was given to this subject, the results were similar; therefore, they have been combined and the average values presented in the table as experiment A. During the third hour there occurred a slight increase in the excretion of most of the urinary constituents, after which there was a corresponding gradual decrease throughout the next three hours. However, these

TABLE 1

Effect of administration of glycine on urinary excretion of nitrogen and sulphur partition products and inorganic phosphorus

(The data are in terms of milligrams per hour of nitrogen, sulphur, or phosphorus, respectively)

EXPERIMENT	TIME OF DAY	URINE VOLUME	TOTAL NITROGEN	UREA	AMMONIA	PREFORMED CREATININE	CREATINE	URIC ACID	TOTAL AMINO ACID	INORGANIC PHOSPHORUS	TOTAL SULFUR	INORGANIC SULPHATE	CONJUGATED SULFUR	NEUTRAL SULFUR
		cc. per hour												
A	7:00 to 9:00	34	362	304	15.6	17.9	0.5	6.3	10.1	11.5	18.1	12.8	2 0	3.2
	9:00 to 10:00	201	474	423	17.2	19.9	0.7	8.8	9.7	9.0	21.8	14.5	2 9	4.4
	10:00 to 11:30	167	424	368	14.6	18.2	0.4	7.4	7.8	9.7	19.0	13.1	1 5	4.4
	11:30 to 1:00	180	377	325	14.4	17.3	0.4	7.0	6.6	12.8	16.7	11.7	1.7	3.2
B	7:00 to 9:00	120	395	326	15.1	18.5	0.3	4.9	7.6	9.3	14.4	11.0	1 0	2.4
	9:00* to 10:00	220	457	379	17.2	19.7	0.5	8.9	18.6	13.0	18.3	13.6	1 4	3.3
	10:00 to 11:00	160	568	414	25.4	19.7	0.7	18.1	81.7	21.2	18.9	14.2	2 2	2.5
	11:00 to 12:00	425	674	556	16.9	19.6	0.4	14.8	54.7	18.6	19.4	14 2	2 4	2.8
	12:00 to 1:00	210	587	520	9.1	18.1	0.3	8.4	24.1	25.5	19.2	14.3	1.5	3.4
C	6:45 to 9:02	23	339	275	14.3	18.9	0.7	5.0	20.5	10.7	12 2	7.9	1.6	2.6
	9:02* to 10:00	142	464	373	15.6	18.0	4.0	7.9	35.1	16.3	13.3	9.1	1.6	2.6
	10:00 to 11:00	93	552	381	25.1	18.4	8.2	14.6	103.7	26.7	15.1	10.7	1.8	2.6
	11:00 to 12:00	405	708	556	21.8	20.4	6.2	19.2	83.4	20.8	18 6	12.7	2.4	3.5

* Twenty grams of glycine administered in 200 cc. of water.

changes, which may have been the result of the establishment of a mild diuresis, were fairly uniform and not large; they constitute a fair representation of the magnitude of the variations to be expected for the particular conditions adopted. In the next experiment (B in table 1) 20 grams of glycine were given for the first time at 9:00 a.m. Thereafter the excretion of nitrogen gradually increased; the total increase in four hours, calculated on the assumption that the basal elimination of nitrogen in the two-hour control period remained constant, was equivalent

to 19 per cent of the nitrogen administered as glycine. Four per cent of the nitrogen which was administered as glycine was excreted as amino acid nitrogen, probably as glycine itself, and most of the remainder was excreted as urea nitrogen. There was no significant change in the excretion of creatinine or creatine; some increase in the excretion of ammonia and a large increase in the excretion of uric acid were noted. The excre-

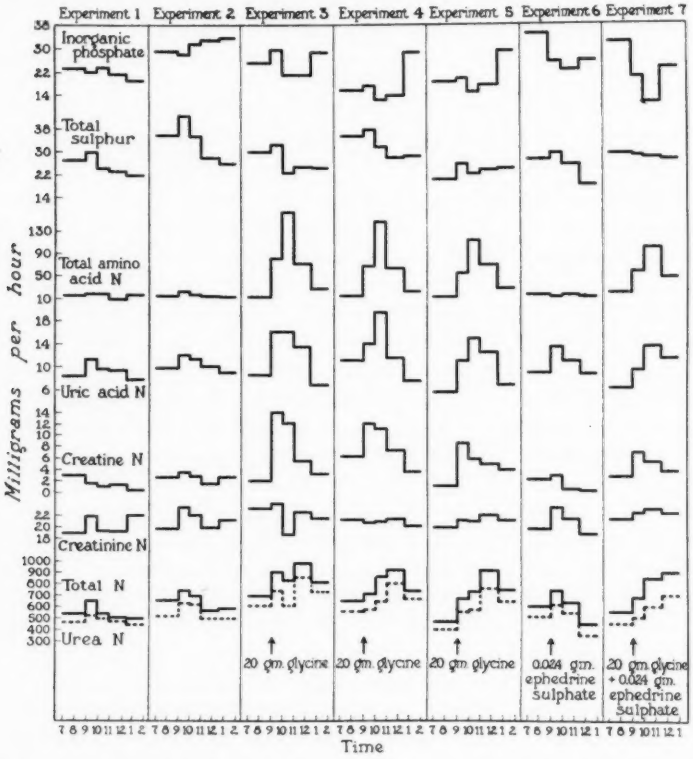

Fig. 1. Curves illustrating the effect of administration of glycine on the excretion of certain urinary constituents.

tion of sulphur and inorganic phosphorus also was definitely increased in this experiment.

Two weeks later, the immediate effect of administration of glycine to the same subject was again studied. The conditions were exactly similar to those of experiment B except that the subject had been taking 20 grams glycine daily during the interim. The response to the administration of 20 grams of glycine (exper. C in table 1) was much the

same as it was in experiment B, except that the elimination of creatine increased enormously as compared with the negligible creatinuria produced by the first dose of glycine. Although the subject did not excrete creatine normally, a creatinuria developed after glycine had been administered for five days, and the results of experiment C illustrate that the elimination of creatine after the administration of glycine, if it occurs at all, can be detected within the first hour after its administration.

A somewhat more extensive series of observations was made on another normal young woman; the results of these are shown in figure 1. The effect of the ingestion of 200 cc. of water at the beginning of each period is illustrated in experiments 1 and 2. In each succeeding experiment water was administered in like manner, and, in addition, glycine or other substance to be tested was administered after a preliminary period of two hours. The administration of glycine (20 grams daily) was begun two weeks after the day of experiment 2, and was continued for one month thereafter. Experiment 3 shows the response to the first dose, experiments 4 and 5 show the response to the regular daily dose one week and one month later, respectively. The daily administration of glycine was then discontinued. Three weeks later, the effect of the administration of $\frac{3}{8}$ grain (0.024 gram) of ephedrine sulphate was investigated (exper. 6), and after another week the effect of the administration of both ephedrine sulphate and glycine (exper. 7) was investigated. It may be seen that this subject, unlike the preceding one, was already excreting small amounts of creatine, and responded to the initial dose of glycine with a marked increase in the excretion of creatine in the hourly specimens of urine. This was likewise observed in each succeeding experiment in which glycine was given. It also may be noted that the excretion of uric acid was increased in each experiment in which glycine was given. In the three experiments in which glycine was administered alone, the increased urinary elimination of nitrogen in five hours accounted for 27, 23 and 43 per cent, respectively, of the nitrogen administered as glycine, while in the experiment in which both glycine and ephedrine sulphate were given, 30 per cent of the nitrogen administered as glycine was excreted within five hours. Most of the increased urinary nitrogen was in the form of urea and amino acids; the increased elimination of the latter corresponded respectively to 9, 8, 7 and 5 per cent of the nitrogen administered as glycine. The curves for sodium, potassium, calcium, and magnesium are not included in the figure as the variations in the urinary excretion of these substances were slight and inconsistent, both in the control series and in the series in which glycine was administered, with the exception of the excretion of magnesium which possibly was slightly decreased after the administration of glycine.

The results of observations on a normal male subject differed in several

respects from those described for normal females. Following the initial dose of 20 grams glycine there was no appreciable excretion of creatine in the hourly specimens of urine. During two weeks in which 10 grams glycine were ingested daily there was practically no excretion of creatine as judged by examination of each twenty-four hour specimen of urine. At the end of this period the response to the ingestion of 20 grams glycine was again investigated, but still no appreciable excretion of creatine in

TABLE 2

Urinary excretion of creatinine, creatine and uric acid: examples of the response to the ingestion of the first dose of glycine as contrasted with the response after some days or weeks of daily ingestion of glycine

(The data are expressed in terms of milligrams nitrogen per hour)

SUBJECT AND DIAGNOSIS	AGE	SEX	PREVIOUS INGESTION OF GLYCINE	CREATININE					CREATINE					URIC ACID				
				Specimens					Specimens					Specimens				
				1*	2	3	4	5	1*	2	3	4	5	1*	2	3	4	5
	years		*days*															
Number 5; myasthenia gravis	40	M.	0	28.3	28.9	29.4	27.4	27.0	2.0	2.3	0.9	1.0	0.7	9.6	12.1	15.7	11.3	11.6
			21	27.2	31.3	32.5	31.0	31.8	1.9	8.2	12.8	9.6	8.2	8.3	12.6	15.3	13.6	12.1
			35	30.9	34.4	34.5	33.3	30.8	5.4	13.2	11.4	9.3	3.6	9.7	14.4	15.7	18.4	10.9
Number 6; myasthenia gravis	40	F.	0	18.2	18.1	16.6	18.2	16.7	9	17	16	13	5	9	16	15	12	7
			21	20.7	16.4	22.0	16.2	24.8	15	23	38	20	8	11	10	22	10	5
Number 7; myasthenia gravis	37	M.	90	31.1	31.9	28.0	29.7	29.1	3	8	6	1	0	7	12	12	9	7
Number 8; myotonia congenita	33	F.	0	19.5	20.9	19.4	20.2		0.1	1.0	1.1	1.3		7.4	10.0	10.2	8.6	
			11	23.3	23.0	21.3	19.6	23.0	3.0	6.4	6.7	4.0	2.7	7.7	12.9	15.9	12.5	11.9
Number 9; chronic exhaustion	38	M.	0	25.0	26.0	25.1	24.7	23.0	1.5	0.9	0.2	0.4	1.0	8.5	11.7	14.3	8.9	8.8
			17	25.4	25.2	25.0	24.8	28.2	0.2	0.7	0.7	0.2	0	9.4	10.2	11.8	8.4	10.4
			42	25.0	30.3	24.5	29.1	24.4	1.8	1.1	1.1	1.5	0.6	8.4	12.9	13.7	10.9	8.8
Number 10; progressive muscular dystrophy	10	M.	0	2.9	2.3	2.9	2.4		11.3	12.6	20.4	10.9		7.3	6.1	7.4	4.2	
			15	3.0	2.8	2.7	2.7		13.1	22.9	17.3	7.6		7.4	9.5	7.3	4.4	
			120	3.3	2.9	2.6	2.6		16.1	21.6	18.6	8.5		7.6	9.7	12.1	4.2	

* At the end of a preliminary period of two hours specimen 1 was collected; each subject then took 20 grams of glycine, except subject 10, who took 10 grams. Specimens 2, 3, 4 and 5 were then collected consecutively, specimens 2 and 3 respresenting one hour each, and specimens 4 and 5 representing one and a half-hours each.

the hourly urine specimens occurred. The increased elimination of nitrogen in five hours on the days when 20 grams glycine was administered was equivalent to 57 and 52 per cent, respectively, of the nitrogen administered as glycine. The increased elimination of amino acids on these days was equivalent to about 10 per cent of the nitrogen administered as glycine.

The response of the various types of patients to the administration of glycine was qualitatively much the same as the response of normal indi-

viduals; the most constant immediate effect was an alteration in the excretion of creatine and uric acid. Representative data relative to the excretion of these substances by six of the eighteen subjects are assembled in table 2. As was observed for normal individuals an increased excretion of creatine immediately following the first dose of glycine may not occur (subject 5), although if there is a preëxisting creatinuria an increase usually does occur (subject 6). After the daily administration of glycine for some days or weeks, a daily creatinuria usually develops, and, concomitantly, the immediate increase in excretion of creatine after the regular daily dose becomes markedly enhanced. The increase in the hourly excretion of uric acid was observed in all experiments with the exception of the first one on subject 10, who was a boy, aged ten years, who had progressive muscular dystrophy. The proportion of the nitrogen administered as glycine which was excreted in five hours was found to vary considerably among the patients, just as was the case among normal subjects.

In all specimens of blood examined after the administration of glycine a moderate increase in the amount of urea and amino acid was demonstrable; the magnitude and duration of these increases were similar to those reported by Folin and Berglund (3), Witts (4), and other observers. No consistent differences in the curves were noted among the various groups of individuals studied. There was no significant increase in the amount of the uric acid in the blood. The increase of the creatine in the blood did not greatly, if at all, exceed the variability of the method for the determination of this constituent.

Comment. The stimulating effect of glycine (and other amino acids) on the hourly excretion of uric acid by normal individuals has been observed by several investigators, notably, Lewis, Dunn and Doisy (5), and Christman and Mosier (6). The contrary results of Zwarenstein (7) have been attributed, by Christman and Mosier, to an unsuspected error in the determinations of uric acid. Quick (8) has emphasized the importance of collection of urine at frequent intervals in studies of the excretion of uric acid, and has pointed out that an initial stimulation may be followed by compensatory decreased excretion, so that examination of twenty-four hour specimens of urine might fail to reveal the real magnitude of the effect of a given stimulating (or depressing) agent. The excretion of creatine following the administration of glycine, if a creatinuria develops, resembles that of uric acid in that the greatest increase usually occurs within two to four hours. Following this, the rate of excretion of creatine decreases, but does not decrease sufficiently to compensate entirely for the early increase (in cases of preëxisting creatinuria in which such compensation would be possible), for, as we have shown (1), the continued administration of glycine in addition to the customary diet

usually results in a net increase in the excretion of creatine that can be detected with certainty in the twenty-four hour specimen of urine. Probably a large part of this increase occurs in the first few hours after ingestion of glycine. It would seem, therefore, that the ability of glycine to provoke a creatinuria in a given case could be tested more simply and with greater certainty by examining a four-hour or five-hour specimen of urine rather than by examining the twenty-four hour specimen of urine. Such a test, if carried out fourteen to sixteen hours after the last meal, as was done in the observations described in this paper, should also represent the effect of administration of glycine on the excretion of creatine somewhat more specifically and with fewer uncertainties attributable to variations in diet than would studies involving examination of twenty-four hour specimens of urine.

SUMMARY

The composition of urine collected at hourly intervals for four to five hours after the ingestion of glycine has been determined for normal individuals and for patients who had myasthenia gravis, progressive muscular dystrophy, or fatigability of indefinite origin. The response of the individuals in these groups was qualitatively similar. In addition to the increased elimination of urea, uric acid, and amino acids to be expected, there usually occurs an increased excretion of creatine. This may be slight or nonexistent the first time glycine is given. If a creatinuria already exists, however, or has been provoked by the daily administration of glycine, the prevailing hourly rate of excretion of creatine will in general be markedly increased when glycine is taken, either for the first time, or immediately following the regular daily dose, as the case may be.

The experiments were conducted fourteen to sixteen hours after the last meal, consequently the results may be taken to represent the effects of glycine more simply and more directly than the results of studies of twenty-four hour specimens of urine obtained from subjects who are receiving their usual diet.

The hourly excretion of the sulphur partition products and the inorganic constituents was not consistently influenced by the administration of glycine.

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COMPARATIVE STUDY OF THE THALAMIC, CEREBRAL AND CEREBELLAR POTENTIALS

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The study of the electrical potentials of the subcortical ganglia seems of interest for two reasons. It may contribute to a better understanding of the mechanism by which these ganglia function and also to the analysis of the cortical potentials. It is known from the work of Gerard, Marshall and Saul (1933) that rhythmic waves of potentials may be picked up from various parts of the subcortex. One has, however, to bear in mind that the cerebral cortex shows a similar type of discharge, and that corticofugal impulses not only pass by or through these ganglia, but also influence them directly. Thus the question arises as to what type of activity the subcortical ganglia show by themselves, if the influence of corticofugal impulses is eliminated. In the present series of experiments this question was studied in regard to the optic thalamus and the cerebellum. An attempt was also made to ascertain the effect of centripetal impulses as well as that of toxins upon the electrical potentials of the optic thalamus. For the sake of brevity, the potentials of the optic thalamus may be called the electrothalamogram (E Th G), those of the cerebral cortex the electrocorticogram (E Co G), and those of the cerebellum electrocerebellogram (E Ce G). Such terms as electrocerebrogram (Prawdicz-Neminski, 1925), or electrencephalogram (Berger, 1929) point to the cerebral hemisphere as a whole and leave undetermined which particular part of the hemispheres is studied.

METHOD. The experiments were performed on cats. The potentials from the surface of the cerebral cortex, of the cerebellum, or from the exposed dorsal surface of the optic thalamus were led off either by two silver wires coated with silver chloride, or by one silver-silverchloride wire, while the indifferent electrode (a silver plate coated with AgCl) was fastened on a splint of the frontal bone in the roof of the frontal sinus. In order to record the thalamic potentials, a metal tube, 7 mm.

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in diameter, was screwed into a trephine hole (center of the hole 8–12 mm. behind the coronal suture, 15–19 mm. lateral to the sagittal suture). This tube contained a hard rubber cylinder with concentric needle electrodes, as described by Adrian and Bronk (1929), or insulated silver wires with bare tips that were coated with AgCl. The nuclei of the optic thalamus were reached at a depth of 15 to 19 mm. Again either the uni- or the bipolar method was used, in the latter case the distance between the wires was usually 3.5 mm. In order to study the effect of a variation in the distance of the electrode tips, a metal plug with a bore of 9 mm. was used, containing a hard rubber cylinder in which two electrodes could be fixed at a distance of 2 mm. and a third electrode at a distance of 4.5 mm. from this pair. The study of regional differences of electric discharges within the optic thalamus was facilitated by the anatomic description of Ingram, Hannett and Ranson (1932) giving the relation of these nuclei to the coördinates of Clarke's stereotaxic apparatus. A unipolar lead was introduced by means of this apparatus. The potentials were recorded by a Sanborn string galvanometer with a three stage amplifier previously described (Spiegel, 1934). The animals were held under ether or dial anesthesia, or were curarized. The site of the electrodes was controlled on frontal sections, in a part of the cases also on serial sections, stained by Weil's method.

Results and comment. The *electrothalamogram* resembles very closely the electrical discharges of the cerebral cortex as described by Berger (1929), Bishop and Bartley (1932), Fischer (1932), Kornmueller (1933), Adrian and Matthews (1934a, b), in that it shows two types of waves, large, slow waves (length, usually from 90–160 msec. sometimes up to 300 msec.), corresponding to Berger's α waves on his electrencephalograms, and small brief waves (length from 10–20 msec.), corresponding to his β waves. (See fig. 1A from a curarized animal.)

Regional differences. Records were taken by the unipolar method from the following nuclei: n. anteromedialis, n. anteroventralis, n. medialis dorsalis, n. ventralis (pars anterior, pars medialis, pars externa, pars arcuata), n. lateralis (pars anterior, intermedia, posterior), pulvinar. Although differences in the records from these various nuclei could be observed, these differences did not seem constant enough to warrant the inference that each thalamic nucleus yields a different more or less typical pattern of potential. If one takes repeated and prolonged records from a given thalamic nucleus, the differences in the electrogram may be as pronounced as the differences between the records from different nuclei.

The oscillations are independent of the respiration and of the rhythm of the heart. They were absent in control experiments on dead animals.

Effect of the distance of the electrodes. If bipolar leads are used, the increase in their distance increases the amplitude of the slow waves (fig.

1B). This is in agreement with observations of Berger (1933a) and of Adrian and Matthews (1934) on the cerebral cortex. These observations do not as yet permit an answer to the question whether the electric discharges of the single cells of the optic thalamus form only brief oscillations or whether cells with a slow type of discharge also exist; it may, however, be inferred that summation phenomena play an important rôle in the genesis of the slow waves. Thus at least a part of the slow waves of the E Th G may find an interpretation similar to that offered by Adrian and Matthews[2] for the slow waves of the E Co G. As to the nature of this summation, one has to bear in mind that simultaneous discharges of single neurons would increase the amplitude, but not the duration of the waves. Summation of small brief waves may, however, induce large slow waves, if the rise in potential of a cell B follows

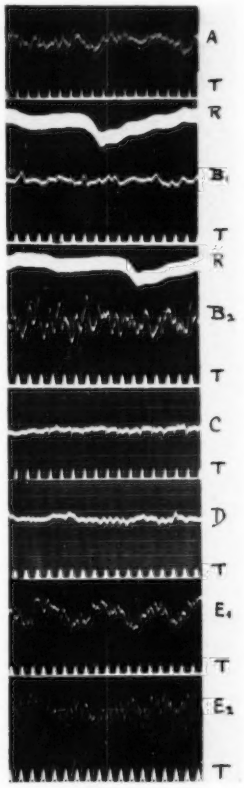

Fig. 1

Fig. 1. Electrothalamograms. 1A. Cat in curare paralysis. Unipolar lead, testing electrode in the nucl. anteroventralis; indifferent electrode on splint of frontal bone; 0.1 mv = 6 mm.

1B. Cat in dial anesthesia, 3 electrodes in the optic thalamus (distance between I and II is 2 mm., between I and III is 4.5 mm.). B_1: Lead I–II, B_2: Lead I–III; 0.1 mv = 6.5 mm.

1C. E Th G. After extirpation of both hemispheres. Superficial ether anesthesia. Bipolar leads (in the nucleus lateralis, pars posterior and the corpus geniculatum mediale); 0.1 mv = 5.6 mm.

1D. E Th G from left optic thalamus after extirpation of the left hemisphere. Bipolar leads; distance between electrodes 3.5 mm. Superficial ether anesthesia.

1E. Effect of strychnine. Cat in curare paralysis. E_1: before the injection, E_2: 5 minutes after intravenous injection of 0.4 mgm. strychnine sulfate per kilo body weight. Unipolar lead, testing electrode in the nucleus lateralis, pars posterior of the left thalamus; 0.1 mv = 5.2 mm. T = time in 0.1 second intervals. R = Respiration.

[2] Adrian and Matthews explain in one paper the genesis of the slow waves in the cortex by a synchronous beat of the cells (1934b) and their extinction on sensory stimulation by the break up of the synchronous action, in another paper they explain the slow waves by an asynchronous activity (1934a). My explanation assumes for the above mentioned reasons an asynchronous excitation of the cells, but in a definite regular succession.

the rise in a neighbouring cell A, before the potential of A has dropped to zero. The same applies to a cell C in relation to B, and to D in relation to C, etc. The anatomic foundation (see Cajal, 1911; Lorente de Nó, 1934) for such a successive stimulation of cells is represented on the one hand by cells with short axons that arborize and terminate within a nucleus, or within neighbouring cortical layers, on the other hand by collaterals of the long axons that transmit impulses from a cell A to the body or to the dendrites of a neighbouring cell B, etc., so that chains of neurons are formed.

Elimination of corticofugal impulses. It proved difficult to destroy by electrocautery the medial surface of the cerebral cortex, and histologic examination showed that the cortex in the bottom of the sulci remained intact, even if the dome of the gyri was destroyed by the cautery. It seemed therefore preferable to remove the hemispheres mechanically. After the hemispheres except the medioventral part of the temporal lobes were extirpated, the electrogram was taken from the anterior or the dorsolateral nuclei of the thalamus the corresponding cortical areas of which were definitely destroyed. Although the amplitude of the waves was diminished (fig. 1C, D) as compared with records from the diencephalon with the cerebral cortex intact, the records still showed waves in a slow rhythm (average 11 per sec.). One can understand that in an acute preparation the activity of the subcortical ganglia is diminished shortly after the decortication. This procedure, however, did not abolish slow waves of a rhythm similar to that observed on the corticogram; one may conclude that such a slow rhythm of electrical discharges may originate in the thalamus independently of the cortex.

Action of toxins. The study of narcotics shows on the one hand that a "cortex anesthetic" such as ether eventually diminishes also the oscillations led off from the optic thalamus; on the other hand it shows that the α-waves of the E Th G may be well preserved in sleep induced by a barbiturate such as dial (fig. 1B), although its locus of action appears to be chiefly in subcortical centers (Fulton, Liddell and Rioch), and although it has a much slighter depressive effect upon the spontaneous electric discharges of the cortex than ether (Bremer, 1936; Derbyshire, Rempel, Forbes and Lambert, 1936). In the deep dial anesthesia, the frequency of the slow waves becomes diminished. If such large doses of dial are injected that the cerebral circulation becomes impaired, then the amplitude of all the oscillations is eventually so diminished that α and β waves can no longer be distinguished. These findings on the E Th G under the action of anesthetics indicate that a distinction between "cortex-anesthetics" and "brain-stem-anesthetics" (Pick and Molitor, 1929) only means that the anesthetics act predominantly but not exclusively upon certain parts of the central nervous system. From a more general point

of view, it seems of interest that quite a considerable degree of spontaneous oscillations may be picked up not only from the cortex (Bartley and Bishop, 1933; Adrian and Matthews, 1934), but also from the optic thalamus, when consciousness is so much diminished that its outward manifestations are abolished. Thus some degree of neural activity may still be maintained in the prosencephalic as well as in the diencephalic centers upon which consciousness depends, even if the state of consciousness is so far decreased that spontaneous movements cease.

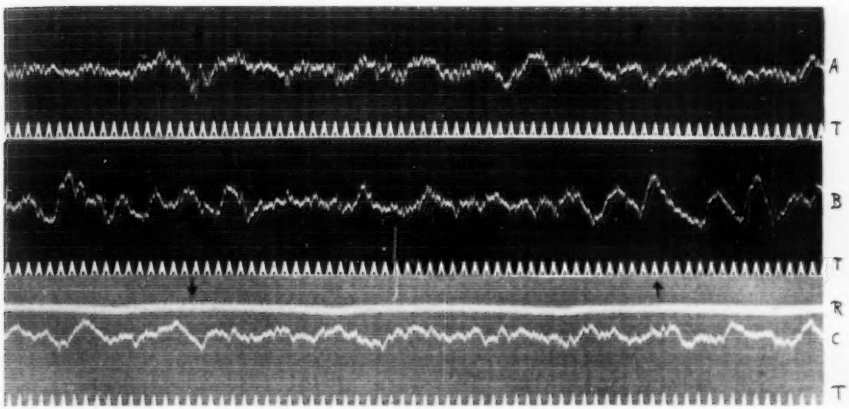

Fig. 2. Effect of sensory stimulation upon the E Th G. A. Pin prick on right foreleg from ↓ to ↑. The pin is fused into a glass holder. Testing electrode in the nucleus lateralis pars posterior of the left optic thalamus (unipolar lead). Cat: Curare paralysis. 0.1 mv = 5.2 mm.

B. Pin prick on right foreleg (from ↓ to ↑). Cat in curare paralysis. Bipolar leads in left thalamus.

C. Stroking of right hind leg with test tube (from ↓ to ↑). Cat in superficial ether anesthesia. Bipolar leads in left thalamus (nucl. lateralis, pars posterior and pars anterior). T = time in 0.1 sec. intervals. R = Respiration.

Also in catalepsy, as produced by injection of bulbocapnine (20–40 mgm. per kilo weight), both types of waves are retained. Intravenous injection of 0.4 mgm. strychnine sulfate per kilogram body weight in curarized animals markedly increases the oscillations of the E Th G (fig. 1E).

Effect of centripetal impulses upon the E Th G. One may note as in the cortical responses, various types of reactions (fig. 2), on the one hand, changes of potential similar to those observed on the cortex by numerous authors (recently Fischer, 1932; Bartley and Bishop, 1933; Kornmueller, 1933; Foerster and Altenburger, 1934), on the other hand a decrease or

disappearance of the α waves, analogous to the decrease observed by Berger (1929, 1933), and Adrian and Matthews (1934a) on the corticogram. The changes of potential may consist of slow deflections of the string forming one or more large waves and/or of an increase in the amplitude of the brief waves. The slow changes in potential are more easily demonstrable with a unipolar lead, the diminution of the α waves with bipolar leads. The increase in the amplitude of the brief waves may be combined not only with the slow changes of potential, but also with the diminution of the α waves. Occasionally one may observe that the stimulation induces the appearance of a group of slow waves.

As to the first type of reaction, i.e., the appearance of large potential waves and the increase of the fast oscillations, Berger's (1935) explanation seems acceptable also in regard to the thalamus. If a sensory impulse enters the cortex or thalamus and increases their activity, the associated change in potentials may become manifested, particularly with the unipolar method, when the cells in the neighborhood of the testing electrode are reached by the impulse. As to the second type of reaction, the diminution of the α waves, Berger assumes that the increase in potential produced in a cortical center by the entrance of centripetal impulses is associated with an inhibition in the cortical areas outside this center. With bipolar leads the decrease in the activity of a large number of cell groups outweighs, according to Berger, the increase of potential in a relatively small area, so that a diminution of the potentials is recorded. There was, however, no indication either in the thalamograms or in the corticograms, that stimulation of a peripheral receptor induces an inhibition in areas more or less distant from the zone of increased potential. Such an inhibition should also appear with a unipolar lead, for instance if one illuminates the eye and places the testing electrode on various parts of the cortex, outside the area striata. Neither on stimulation of the retina, nor of other receptors (cochlea, skin) could such an inhibition be observed. If one considers most of the slow waves summation effects induced by successive excitation of cells in neuron chains, it seems conceivable that interference phenomena will be produced that interrupt the normal cycle; the slow wave will consequently be diminished, although the single brief waves may be increased. Occasionally it may occur that the afferent impulse reaches a link of the chain simultaneously with the impulse that circulates in the chain or after recovery from previous discharges so that a slow wave is reinforced, or the formation of a new slow wave is induced.

Effect of elimination of afferent impulses. Transverse section of the midbrain was performed, while the carotid arteries were clamped during this operation only. It is rather difficult to obtain electrical discharges from the optic thalamus after this operation. In some cases, however,

oscillations still appeared, particularly when the ganglion was in a state of hyperexcitability after injection of strychnine; in this case, even an increase of the oscillations could be observed immediately after the decerebration, apparently due to a direct stimulating effect of the section, subsiding after a few minutes. Usually, however, the oscillations show, when appearing at all, a smaller amplitude and also a smaller frequency than before the operation. Groups of waves may appear and be interrupted by periods of decreased activity (fig. 3). If one bears in mind that the impairment of the circulation, of the oxygen supply of the ganglion and of the temperature regulation depresses the activity of the ganglion cells, the positive cases (with retained oscillations) seem of greater significance than the negative ones. It may be inferred that a certain degree of cellular activity may persist in the optic thalamus under favorable conditions after elimination of afferent impulses. This does not, of course, exclude that tonic, continuous afferent impulses normally play an important part in maintaining the activity of the thalamus that is manifested

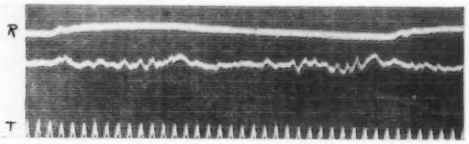

Fig. 3. Electrothalamogram after transverse section of the midbrain. Bipolar leads (right thalamus; nucl. lateralis pars posterior and pars anterior). Spontaneous respiration (R); 0.1 mv = 6.5 mm. T = time in 0.1 sec.

by the α waves. We have here a further analogy to the cerebral cortex, since Bremer (1935) found slow waves preserved in the corticograms after transverse section of the midbrain. Additional severance of the olfactory nerves and elimination of optic impulses by closing the lids did not change these results on our thalamograms.

In order to study the *E Co G after severance of the thalamocortical* connections incisions were made into the hemisphere with a wide, double-edged knife, 8 to 9 mm. behind the cruciate sulcus, and the potentials of the anterior and posterior sigmoid gyrus were recorded as soon as possible after the operation. In the most successful experiments, the cortical area under study was completely separated from the optic thalamus; the only connection with cortical areas behind the incision (parietal, occipital, temporal lobe) was a small strip of cortex on the medial and ventral circumference of the hemisphere; there was no connection with the opposite hemisphere, since the incision was made through the anterior end of the corpus callosum. α waves could still be picked up (fig. 4) from the sigmoid gyri which were thus isolated. They often showed

changes similar to those in the thalamograms after the transverse section of the midbrain, such as diminution of the amplitude and of the frequency, periodic appearance and disappearance, apparently mainly due

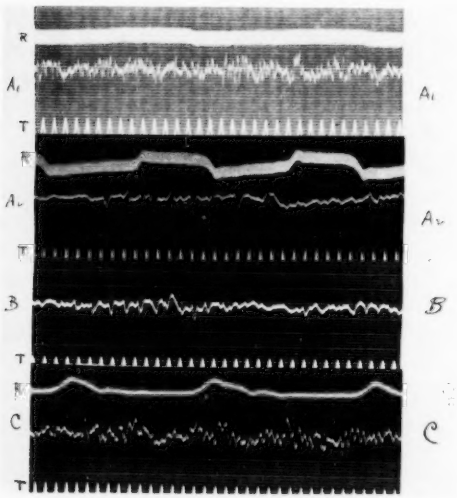

Fig. 4

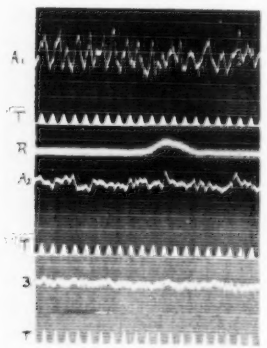

Fig. 5

Fig. 4. Electrocorticograms. A_1 from the posterior sigmoid gyrus before, A_2 after incision into internal capsule, 9 mm. behind the cruciate sulcus. (Ether anesthesia.)

B. From the right posterior sigmoid gyrus after incision into this hemisphere destroying the connections with the occipital lobe and the posterior part of the temporal lobe. (Dial anesthesia.)

C. One lead anterior, one lead posterior to the left cruciate sulcus. Incision into this hemisphere 8 mm. behind this sulcus that destroys the internal capsule, the left caput nuclei caudati, the left half of the corpus callosum in its most anterior part, and separates the frontal lobe nearly completely from the remaining brain. Only a small strip of cortex on the medial and ventral circumference connects the frontal lobe with the posterior part of the brain. R = Respiration. 0.1 mv = 6 mm. T = time in 0.1 sec.

Fig. 5. Electrocerebellograms. A. Bipolar leads from the posterior part of the vermis. A_1 before, A_2 after the removal of the cerebral hemispheres and the optic thalamus and section of the inferior peduncles (distance between the electrodes 3 mm.) 0.1 mv = 6 mm. R = Respiration.

B. Bipolar leads from the lobus anterior. Decerebrate cat. Severance of all cerebellar peduncles. T = time in 0.1 sec.

to the impairment of the cortex by the neighbouring injury. The persistence of the α waves in the nearly completely isolated frontal lobe shows, in agreement with the views and observations of Berger (1933a),

Bartley and Bishop (1933), and Dusser de Barenne and McCulloch (1936), that these waves may originate in a rather limited cortical area; it furthermore shows that impulses from other cortical areas as well as from the optic thalamus can be dispensed with.

The experiments furnished no evidence in favor of the assumption (Berger, 1933b) that thalamic impulses have a continuous inhibitory effect upon the cortical discharges, since increase of the cortical potentials was not observed after severance of the thalamocortical pathways. This does not exclude, however, the possibility that such inhibitory impulses exist, since the injurious mechanical effect of the operation, the diminution of the cerebral circulation, impaired the cortical activity after the severance of the thalamocortical pathways.

The *electrocerebellogram* was first recorded from the vermis and the hemisphere with the central nervous system intact, after the surface of the cerebellum was exposed by trephining the occipital bone. The records showed not only rapid oscillations at rather high frequencies as observed by Adrian (1935), but there appeared also, at least in some of the records, slow waves (fig. 5A). This is in agreement with observations of Foerster and Altenburger (1934) in the human cerebellum. The same preparation that first gave rise to rapid waves only could at a later stage of the experiment show also slow waves. Both types of waves could still be led off from the dorsal surface of the cerebellum after decerebration was performed by transverse section behind the mid-brain, and the parts of the brain cranial to the section were removed. After the cerebellar peduncles were severed in such a decerebrate preparation, the amplitude of the oscillations markedly diminished, but slow and fast waves still could be distinguished.

Concluding remarks. We have seen that potential waves similar to those from the cerebral cortex can be led off from the optic thalamus and, at least in some cases, from the cerebellum, even in decorticated animals. In other words, the oscillations recorded in the electrocorticogram are not at all specific for the cerebral cortex.[3] Slow waves of potentials may arise not only in the cortex but also in the subcortical ganglia under study, independently of other parts of the brain and, at least to

[3] Berger is inclined to regard the α waves as phenomena accompanying those cortical processes that are called psychophysic, because they may under some circumstances be accompanied by phenomena of consciousness. One has to bear in mind, however, that similar discharges may be picked up from various parts of the subcortex after extirpation of the hemispheres and also in rather deep anesthesia. It is quite possible that the fundamental process accompanied by the α waves represents a prerequisite for the appearance of psychic phenomena, such as Wundt's apperception waves. The question however, remains unsolved what changes this fundamental process has to undergo, or what additional processes are necessary in order that psychic phenomena appear.

a certain degree, also of centripetal impulses. This latter fact indicates that, under favorable conditions, stimuli originating in the metabolism of the cells or transmitted through the blood stream may elicit a "spontaneous" (autogenous) activity, not only in the spinal cord (Brown 1914) and in the respiratory center, but also in higher parts of the mammalian central nervous system.

SUMMARY

1. The oscillations recorded in the electrocorticogram (E Co G) are not specific for the cerebral cortex. The electrothalamogram (E Th G) shows slow and rapid waves quite similar to those of the E Co G. The electrocerebellogram (E Ce G) may show rapid waves only or also slow waves (experiments on cats).

2. The slow waves of the E Th G may persist after elimination of corticofugal impulses or after transverse section of the midbrain; the amplitude and frequency of these waves, however, usually are diminished after these operations.

3. With bipolar leads, increase of the distance of the electrodes increases the amplitude of the slow waves of the E Th G.

4. The depressor action of ether is not limited to the cortex, but also noticeable in the E Th G; the typical form of the electrothalamogram is preserved in sleep, as induced by dial; also in bulbocapnine catalepsy both types of waves are retained. Injection of strychnine on curarized animals increases the oscillations of the E Th G.

5. Sensory stimulation may induce slow deflections of the string, increase of the brief oscillations, as well as a diminution of the slow waves of the E Th G.

6. The electrocorticogram picked up from a circumscribed cortical area (e.g., sigmoid gyri) still shows slow waves after severance of the thalamocortical pathways and of nearly all connections with the rest of the cortex.

7. The oscillations of the electrocerebellogram persist after transverse section of the midbrain; they are diminished but still present after severance of the afferent cerebellar tracts.

8. Summation of rapid waves due to successive stimulation of cells in neuron chains may at least partly explain the genesis of the slow waves. The effects of sensory stimulation are explainable on the one hand by appearance of action potentials of the cells reached by the centripetal impulse, on the other hand by the interference between the sensory impulse and the wave circulating in the resting neuron chain.

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CHEMICAL AND HISTOLOGICAL CHANGES IN DENERVATED SKELETAL MUSCLE OF THE MONKEY AND CAT

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Simple atrophy of disuse (1) contrasts sharply with the atrophy plus degeneration which occurs after severance of the motor nerve. Our objective was to determine whether the degenerating muscle showed demonstrable changes in water, nitrogen, lipid and phosphorus content, and whether the histologic changes could be correlated with the chemical.

MATERIALS AND METHODS. Seven young Macacus rhesus monkeys were used. Under ether anesthesia, 5 cm. of the right sciatic nerve were removed. The femoral nerve was severed in the inguinal region, to paralyze the other leg muscles and prevent passive motion of the gastrocnemius-soleus. The extremity thus paralyzed was placed in a plaster of Paris cast, applied from above the pelvis to beyond the toes. Before applying the cast, the limb was padded very thoroughly to prevent ischemic complications. The cast was fixed with the limb in a position of slight flexion at the knee, thereby minimizing stretching of the gastrocnemius-soleus. The cast was applied chiefly for protection, inasmuch as preliminary studies showed that slight trauma to a denervated extremity readily produced ulcerations and secondary infections. At designated periods of 1, 2, 3, 6, 8, 10 and 12 weeks, the gastrocnemius-soleus muscles were excised from both lower extremities. Those of the normal left leg served as controls.

Immediately following excision, the muscle group was weighed and then bisected longitudinally. One portion was used for histologic study and the other for chemical analyses. For histologic studies, the sections were stained by a modified Ranson pyridine silver, the hematoxylin and eosin, and Van Gieson methods.

Water content was determined by desiccation over sulphuric acid at reduced pressure (30 mm. Hg). Desiccation was continued until constant weight of the specimens was obtained. Nitrogen was determined as total nitrogen by the Kjeldahl method. All determinations were in duplicate. Phosphorus was determined as total phosphorus by a modification of the method of Fiske and Subbarow (2). Duplicate samples of aliquots of the wet ash were used.

For the studies of lipid content, it was found that sufficient material was not available from the monkey specimens. A separate series of cats, therefore, was used, employing the same procedure of denervation as above. Lipids were extracted with acetone and ether (24 hours each) in a Soxhlet apparatus. The acetone-ether extracts for each specimen were then combined, evaporated to dryness, and the residuum weighed directly.

RESULTS AND DISCUSSION. The progressive histologic changes which occur following denervation of striated muscle have been described previously by others. The subject has been carefully reviewed by Stier (3) and by Jamin (4).

In 1935 Tower (5) published the results of a careful restudy of denervation atrophy. Our observations on the changes in denervated gastroc-

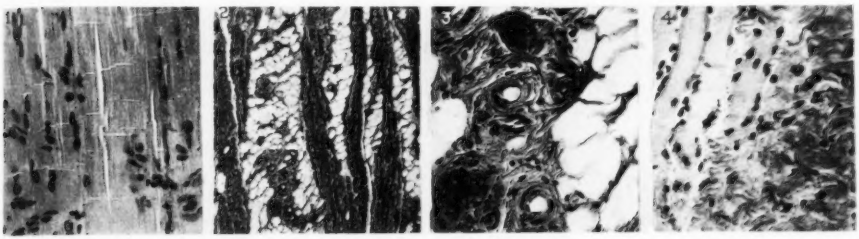

Fig. 1. (*1*) Monkey muscle one week after nerve section, showing multiplication of sarcolemmal and muscle nuclei and beginning fading of cross-striations. (*2*) Monkey muscle six weeks after nerve section showing considerable replacement of muscle fibers by fibrous tissue and fat. The increase in nuclei is striking and capillary dilatation may be seen. (*3*) Monkey muscle six weeks after nerve section showing fibrosis of the walls and endothelial hypertrophy of the small intra-muscular blood vessles. (*4*) Monkey muscle twelve weeks after nerve section, showing marked fibrosis of degenerated muscle fibers. A few residual segments of muscle fibers undergoing transformation into fibrous tissue may be seen.

nemius-soleus muscles of monkeys parallel those of Tower on the interossei of cats, with a few exceptions which will be referred to later in this discussion.

The earliest changes which occur in skeletal muscle following nerve section were established by Krauss (6) in 1888. The muscle fibers become narrower and the striations slightly paler than normal. The striking feature, however, is the increase in nuclei. Long rows of vesicular nuclei appear under the sarcolemma and also within the muscle cells. This process of nuclear multiplication, good examples of which are not found in the literature, is illustrated in figure 1, *1*.

At the end of two weeks, more advanced changes occur. Longitudinal striations are apparent as a result of cleavage between small bundles of myofibrillae. In the present study, it was found that even at this early

stage a few fibers showed destruction of the homogeneous cross-striation pattern, although in most of the fibers this was still preserved. In some sarcomeres, the substance responsible for the dark striations has been replaced by a coarsely granular mass implying a breakdown of portions of the myofibrils. Such muscle fibers show marked distortions as a result of the contraction of intact sarcomeres of a partially degenerated fiber. These findings deserve special comment in view of Tower's (5) conclusions that the early changes in denervated muscle (which she designates "atrophy" in contrast to "degeneration" which occurs later), involve the sarcoplasm and nuclei of the muscle fibers, and not the myofibrillar structure. She has stated that this indicates a dependence of nuclear and sarcoplasmic structures on innervation—a dependence of which the specifically contractile element, the myofibril, is free. She did find, however, in a few specimens, that some muscle fibers were replaced by sheets or fascicles of fibers of one-half or one-third the normal diameter. In these the longitudinal fibrillae were uncommonly prominent and the cross-striation pale (p. 12). Our findings, however, do not warrant the conclusion that the sarcostyles or myofibrillar elements are independent of innervation. It is true that the sarcoplasm is apparently the first to be involved. This loss of sarcoplasm no doubt accounts for the shrinkage of the muscle fibers, a feature which was stressed in the study of simple disuse atrophy with innervation intact (1). In nerve section atrophy, a similar loss of sarcoplasm occurs early, associated with inactivity of the paralyzed muscle.

Six weeks after denervation, degeneration of the myofibrils is very prominent. Striations are no longer visible in many fibers. The muscle cells have been converted into granular structures in which vacuoles may be seen as evidence of severe breakdown. From the histologic standpoint, it appears that the metabolism of denervated muscle is considerably altered. This will be referred to in relation to the chemical findings.

With the degeneration of the muscle fibers, a reaction on the part of the fibrous tissue elements becomes marked (fig. 1, *4*). This fibrosis is a characteristic feature of the later stage of denervation atrophy, designated by Tower "fibrotic de-differentiation." Associated with fibrosis, the presence of fat becomes conspicuous (fig. 1, *2*). From histologic studies it would appear that fat is increased in this type of atrophy. Heretofore, it has been assumed that there is a definite increase of fat as a result of either fatty degeneration or infiltration. Our chemical findings do not support this assumption.

The changes in the intramuscular nerve endings constitute an important feature of nerve section atrophy. Degeneration occurs in both the motor nerve endings and the sensory end-organs very soon after nerve section, so that by the end of one week, only occasional remnants may be seen

scattered throughout the denervated muscle. The nature of these early changes in the motor nerve endings in the monkey has been described in a previous paper by one of us (H. C.) (7). The changes in the muscle spindles following nerve section were carefully described by Tower (8). Associated with the acute degeneration of the extra-fusal fibers, similar changes occur in the intra-fusal elements. The spiral organ of Ruffini at the equatorial region and the motor end-plates at the polar regions disintegrate early. In the late stages, the spindles as well as the extra-fusal fibers are replaced by fibrous tissue.

Changes also occur in the intramuscular blood vessels. There is an increase in capillaries, and the small intramuscular blood vessels show

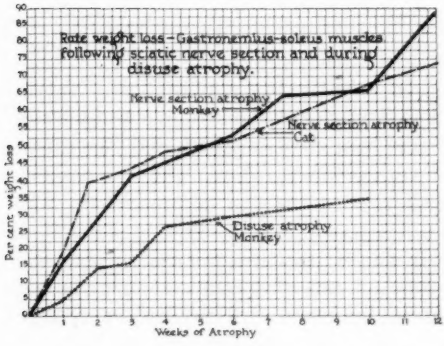
Rate weight loss - Gastrocnemius-soleus muscles following sciatic nerve section and during disuse atrophy.
Nerve section atrophy Monkey
Nerve section atrophy Cat
Disuse atrophy Monkey
Per cent weight loss
Weeks of Atrophy

Fig. 2

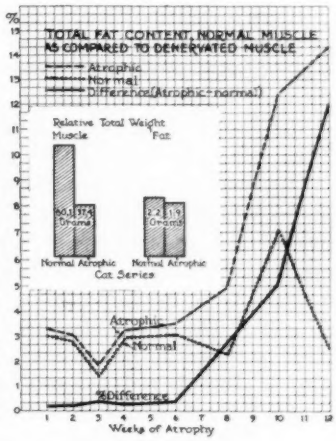

Fig. 3

hypertrophy of the endothelium and an increase in their fibrous tissue structure (fig. 1, *3*).

Within one week after nerve section, 14.6 per cent loss in weight occurred in the denervated gastrocnemius-soleus muscle comparing it with the normal opposite extremity. Atrophy continued at a rapid rate, and by the end of twelve weeks there was an 88 per cent loss in weight. The progress of the wasting process may be seen in figure 2. The rate of weight loss in the separate series of cats used primarily for lipid determinations compares very closely with that of monkey muscle. The curve of weight loss obtained in simple disuse atrophy, reported in a previous study, is included in this chart for comparison.

In attempting to correlate the histological and chemical data, an apparent discrepancy appeared in the lipid content. The histologic appearance

seems to show fatty changes in the region of the denervated muscle fibers and would be interpreted as an actual increase of fat. The determination of total lipids, however, fails to show an actual increase.

Rumpf and Schumm (9) reported the fat content of atrophic human muscle tissue in a case of polyneuritis to be fifteen times that of the normal. Grund (10), producing denervation atrophy in dogs, reported a rise in the ether-soluble fraction of fat from 6 to 17 per cent. Chen, Meek, and Bradley (11), recalculating Grund's figures upon the basis of weight loss of the denervated specimens, interpreted this to mean a 300 per cent increase in fat, inasmuch as the total weight loss of the atrophic specimens was seldom as much as 50 per cent. They further state that this seems to establish the fact that there is an actual increase in fat.

Audova (12) found that fat accumulated in muscle tissue undergoing atrophy following denervation and tenotomy. The data indicate the accumulation of fat was slow at the beginning, becoming more rapid in the later stages. The fat-free dry substance, however, diminished rapidly at first and at a slower rate in the later stages, indicating that the fat increase is probably not at the cost of the disintegrated protein.

The results obtained in the present investigation confirm this observation. However, when the total lipid contents of the atrophic and normal specimens from the same animal were compared, it was found that there was little difference in the values. On a percentage basis, the lipid content appears to be increased, but this is due to the loss of protein through atrophy. The proper conclusion to be drawn from the finding is not that there has been an actual increase in the fat content, but rather that the adipose tissue in striated muscle does not enter into the atrophic process. Figure 3 shows the curve of total lipid content expressed in per cent of muscle weight. That this increase is relative is demonstrated by the block figure in this chart.

Lipschutz and Audova (13) showed a slight but constant increase in the water content of atrophic muscle tissue following both denervation and tenotomy. Chen, Meek, and Bradley (11), however, in a large series of rabbits, found no alteration in the water content. Our findings confirmed the latter work only when simple desiccation was employed. When water was extracted by acetone, however, evidence of a change in the state of hydration of denervated striated cat muscle was seen. After twelve weeks' atrophy, the water content of the atrophic tissue was found to be 12.8 per cent lower than the respective control specimen. It may be that treatment of the muscle tissue with acetone, which is an histologic fixative, results in a rearrangement of the protein structure with a consequent alteration in its state of hydration.

Table 1 gives the values for water in normal and denervated monkey muscles as determined by desiccation over sulphuric acid under reduced vapor pressure. The variations are slight and occur in both directions.

Table 2 shows the changes in water content in denervated cat muscle when acetone was used in addition to simple desiccation.

No alteration in the percentage of total nitrogen, as determined by the Kjeldahl method, was obtained (table 1). Since this procedure determines only total nitrogen and gives no information as to the partition thereof, changes may have occurred which were not detected. It is significant, however, that despite the marked histologic differences between atrophied muscle tissue resulting from immobilization and from nerve section, neither type of atrophy showed any change in the relative proportion of total nitrogen present. This observation does not preclude the

TABLE 1

Water, nitrogen and phosphorus determinations on normal and denervated muscles (monkey)

WEEKS	SPECIMEN*	WATER	N PER GRAM MUSCLE SAMPLE (DRY)	TOTAL P PER GRAM OF MUSCLE (DRY)
		per cent	*grams*	*grams*
1	N	75.39	0.1365	0.00791
	A	74.55	0.1348	0.00665
2	N	75.84	0.1324	0.00793
	A	75.85	0.1323	0.00674
3	N	75.52	0.1320	0.00792
	A	75.45	0.1357	0.00673
6	N	74.25	0.1335	0.00793
	A	75.58	0.1341	0.00691
8	N	73.57	0.1344	0.00790
	A	75.30	0.1349	0.00684
10	N	73.51	0.1337	0.00795
	A	73.48	0.1351	0.00677

*N = Normal.
A = Atrophied.

possibility that qualitative changes in the protein may have occurred. Chen and his co-workers attempted to determine whether there was any difference in the rate of autolysis of atrophic muscle protein as compared with that of normal muscle. They concluded that denervated muscle tissue manifests increased digestibility. On the basis of amino acid production after ten days of post-mortem analysis, this was found to be almost 100 per cent greater than the normals.

The denervated muscle tissue was found to have a lower phosphorus content than the normal and to suffer most of this loss during the first week. After the first week, the phosphorus content remained approximately stationary (table 1). If the replacement of normal muscle by

connective tissue (with its lower phosphorus content) accounted for the decrease in the total phosphorus, one would expect to find the loss of phosphorus progressive. Such was not the case. The decrease occurred by the end of the first week of denervation and did not progress, as the atrophy became more marked. A more logical deduction would be that since phosphorus compounds and muscle contraction are so closely interrelated, a cessation of function resulted in rapid loss of phosphorus.

TABLE 2

Water and lipid content of denervated muscle (cat)

WEEKS	SPECIMEN	TOTAL WEIGHT LOSS (EXTRACTION PLUS DESICCATION)	LIPID	WATER
		per cent	*per cent*	*per cent*
1	N	80.3	3.07	77.23
	A	79.9	3.31	76.59
2	N	81.8	2.81	78.99
	A	82.4	3.08	79.32
3	N	79.7	1.39	78.31
	A	80.6	1.71	78.09
4	N	81.4	2.91	78.49
	A	80.9	3.22	77.68
5	N	79.7	3.11	76.59
	A	79.3	3.49	75.81
8	N	79.5	2.31	77.19
	A	78.9	4.90	74.00
10	N	79.8	7.15	72.65
	A	81.7	12.34	69.36
12	N	81.2	2.40	78.8
	A	80.4	14.40	66.0

In 1934, Bloor and Snider (14) reported a study of the relationship of essential lipid (phospholipid and cholesterol) content of tissue to physiologic activity. It was found in mammals and birds that the phospholipid content of muscle is higher in the more active muscles. The converse of this was borne out by the phosphorus determinations on the denervated monkey muscle. With atrophy, the total phosphorus content, necessarily associated with the phospholipin content of the muscle, decreased.

CONCLUSIONS

1. Nerve section atrophy is more rapid than simple disuse atrophy. Obvious quantitative and qualitative changes occur within one week, and as much as 88 per cent loss of weight may occur within 12 weeks.

2. The process of degeneration of muscle fibers involves disintegration of both the sarcoplasm and sarcostylic elements. The earliest changes consist of nuclear multiplication, narrowing of the individual fibers, and fading of the cross-striations. This is soon followed by granular and vacuolar degeneration with replacement by fibrous tissue.

3. The intramuscular nerves and their endings, both motor and sensory, undergo early degeneration, and are rarely observed after two weeks following nerve section.

4. The intramuscular blood vessels react by fibrosis of the walls and endothelial hypertrophy.

5. These degenerative changes occur unequally throughout the muscle bundles, and even after 12 weeks' denervation, some striated fibers persist.

6. Evidence of attempts at regeneration, such as an increase in sarcolemmal and muscle nuclei and presence of mitosis, is characteristic.

7. The analyses of the water content of atrophic muscle tissue were not in full agreement with those reported by other investigators. Simple desiccation at reduced vapor pressure showed no alteration in water content of muscle undergoing atrophy. Extraction with acetone followed by desiccation as above removed more water from the muscle in the later stages of denervation atrophy than from the normal control.

8. No changes from the normal were obtained in the total nitrogen determinations of the atrophic muscle tissue.

9. The original amount of lipid present in a given muscle was found to remain intact during the course of the atrophic process.

10. The total phosphorus of muscle decreases within a week following denervation. The loss is not progressive after the first week.

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THE EFFECT OF AN ANTERIOR HYPOPHYSEAL EXTRACT UPON THE SERUM CALCIUM AND PHOSPHORUS[1]

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The parathyroid glands play a decisive part in the maintenance of the normal physiological level of serum calcium. It is also well known that the thyroid gland influences the rate of excretion of calcium, although it does not affect the level of serum calcium. Other endocrine organs may influence the concentration of calcium in the blood serum, but apparently do not exert the profound effect upon the serum-calcium level which is attributed to the parathyroids. Studies on these endocrine glands are lacking in the general unanimity of results which has attended investigation of the parathyroids. Conclusions based upon such conflicting experimental evidence cannot be accepted without considerable reservation. Zwarenstein (1), who recently summarized the available data on this point, concludes that the mammalian adrenal cortex and pancreas probably alter the level of serum calcium in certain circumstances. Ablation of the adrenal glands is said to be followed by a rise in serum calcium, whereas injections of chemically crude adrenal cortical extracts are associated with decreases in the serum calcium level. The injection of insulin may result in an increasing serum calcium which is secondary to changes in the blood phosphate level; and Marenzi and Gerschman have found that the blood calcium of dogs decreases after pancreatectomy (2, 3). The literature (1) also suggests that the mammalian gonads and thymus may alter the serum-calcium level to some extent.

Recent studies on the physiology of the anterior hypophysis have disclosed that it also may take part to some extent in the regulation of calcium metabolism and serum-calcium level. Most investigators are agreed that the parathyroid glands show pathological changes after hypophysectomy. As early as 1920, P. E. Smith (4) demonstrated atrophy of the parathyroids

[1] This is No. XXV of the series entitled "Studies of Calcium and Phosphorus Metabolism."

in tadpoles after ablation of the anterior hypophysis. Some years later, Koster and Geesink (1928) and Koster (1930) reported that the parathyroids of hypophysectomized dogs were smaller than those of normal control animals (5). White (1933) suggested that such atrophy in hypophysectomized rabbits probably consisted of a decrease in the size of individual parenchymal cells (16). More recently (1936), Houssay has summarized the data on a relatively large number of dogs which were hypophysectomized in his laboratory over a period of years. In approximately two-thirds of the animals he found characteristic regional degenerative lesions which appeared in the parathyroids between 5 and 14 days after removal of the anterior hypophysis (2).

Houssay, Marenzi, Gerschman and Mazzocco have repeatedly reported that the blood-calcium level of dogs is not significantly altered by hypophysectomy (2). Similarly, Wallace has reported normal values for serum calcium of hypophysectomized rats (7). Koster and Geesink, on the other hand, have found the serum calcium of three hypophysectomized dogs to be lower than that of their control animals (5). Data published by Riddle and Dotti indicate that a slight decrease in serum calcium occurred in several of their hypophysectomized pigeons (8). The most striking of all decreases in serum calcium have been consistently found in the hypophysectomized amphibian, *Xenopus laevi*, by Hogben, Charles and Slome, Charles and Shapiro and Zwarenstein (1). Pugsley and Anderson have shown that hypophysectomized rats show a negative calcium balance which disappears if the animals are injected with anterior pituitary substance (9). It would appear from these studies on hypophysectomized animals that the parathyroids are generally the seat of pathological alterations which may or may not find expression in a change of the level of serum calcium.

Parenchymal hypertrophy of the parathyroid glands has been reported in rabbits (Hertz and Kranes, 1934), rats (Anselmino, Hoffman and Herold, 1933) and dogs (Houssay, 1936) after their injection with anterior pituitary extracts (for references see (2)).

These histological changes are apparently paralleled by an increase in the values for serum calcium. Friedgood[2] found that daily injections of an alkaline extract of the anterior hypophysis resulted in a significant increase in the serum-calcium level of rats and guinea pigs after 6 to 9 days of treatment (10). All of the experimental and control animals were studied simultaneously under the same environmental and dietary conditions throughout these observations.

Anselmino, Hoffman and Herold have reported that such increases in the serum calcium of rats start within 3 hours after a single adequate dose

[2] Observations made during July, 1933, with the coöperation of Dr. D. H. Shelling.

of anterior hypophyseal extract, and do not occur in dogs and rats which are parathyroidectomized previous to injection (2). Marenzi and Gerschman have confirmed the fact that an anterior hypophyseal extract significantly increases the serum calcium of normal dogs (2, 3). Subsequently, more confirmatory data have appeared in the literature, but no conclusions can be drawn from them because of the limited number of animals upon which they are based. Marenzi and Gerschman have also found that their extract does not increase the serum-calcium level of parathyroidectomized dogs (3).

In contrast to these mutually supporting observations, Hogben and Charles have reported that a rapid decrease of the serum-calcium level occurs in rabbits within 4 to 12 hours after injections of a crude anterior hypophyseal extract (11). Their experiments were continued for a maximum of only 19 hours. Similarly, Dixon found no changes in the serum calcium of rats which were injected for 5 days with an anterior hypophyseal extract that showed well-marked gonadotropic properties (12). His series included only three experimental animals and one control.

The importance of this phase of the problem and the lack of complete uniformity of results made it advisable to repeat the previous experiments with guinea pigs on a more extensive scale. The serum-calcium determinations were done at the Huntington Memorial Hospital in Dr. J. C. Aub's laboratory.

EXPERIMENTAL PROCEDURE. Thirty-four guinea pigs, weighing between 340 and 449 grams, were placed upon a diet consisting of leafy vegetables, carrots, and a mixture of alfalfa meal and oats. Cabbage was excluded from the diet because of the effect which it is said to have upon the serum-calcium level of rabbits (12). Fifteen of these animals served as controls and were sacrificed at various intervals during the experimental period. The remaining 19 animals were injected daily for 9 days with 1 cc. of an alkaline extract of the anterior hypophysis[3] and were autopsied on the 10th day of the experiment. After a preliminary period of fasting for 18 to 20 hours, the animals were etherized and their blood withdrawn directly by cardiac puncture. The serum calcium and phosphates were then determined according to the methods described by Fiske and Logan, and Fiske and Subbarow (13, 14).

[3] We are indebted to E. R. Squibb & Sons for the extract used in these experiments.

RESULTS.

SEX OF GUINEA PIG	UNTREATED CONTROL GROUP		SEX OF GUINEA PIG	EXTRACT-TREATED GROUP	
	Serum calcium	Serum phoshate		Serum calcium	Serum phosphate
	mgm. per cent	*mgm. per cent*		*mgm. per cent*	*mgm. per cent*
F.	11.9		M.	13.5	
F.	11.7	7.9	F.	13.3	6.8
F.	11.7	6.5	M.	13.2	
M.	11.6	6.7	F.	13.0	8.3
M.	11.5	7.9	M.	12.7	
M.	11.4	7.7	F.	12.6	8.9
F.	11.2	8.0	F.	12.3	6.9
M.	11.0	7.3	F.	12.2	
F.	11.0	7.3	F.	11.7	7.1
F.	10.9	8.9	F.	11.7	9.4
F.	10.8		M.	11.7	
F.	10.7		M.	11.7	7.3
M.	10.7		F.	11.6	7.2
F.	10.6		M.	11.5	
F.	10.5	6.5	F.	11.2	7.6
			M.	11.2	
Average....	11.1	7.5	M.	11.2	7.1
			M.	11.1	
			M.	10.6	
			Average....	12.0	7.7

These data show that there were no significant changes in the level of serum phosphates as a result of extract therapy, but the serum-calcium level was affected. Comparison of the averaged data for these two groups of animals does not convey the extent of the shift which takes place in serum-calcium values of animals injected with an anterior hypophyseal extract. However, the difference between the two means (0.9) is statistically significant, being more than four times its standard deviation. The table which follows also brings out this point satisfactorily.

EXPERIMENTAL CONDITIONS	NUMBER OF ANIMALS	
	Serum calcium less than 11.0 mgm. per cent	Serum calcium greater than 11.9 mgm. per cent
Control group, 15 animals	6	0
Extract-treated group, 19 animals	1	8

These results confirm Friedgood's previously published data for guinea pigs and may be construed as further evidence for the belief that an extract of the anterior hypophysis can raise the level of serum calcium. In this

connection it is of interest to point out that the guinea pig is said to be highly resistant to the effects of the parathyroid hormone. Bodansky, Blair and Jaffe found that the response of the guinea pig to a single dose of 100 to 200 units of parathyroid hormone depends upon the age of the animals and their state of nutrition (15). Young guinea pigs, fasted 60 hours, responded to the extract with an increase of the serum calcium from 10.5 to as much as 15 mgm. per cent (24 to 36 hours after injection), whereas old or unstarved animals were apparently unaffected. The animals in our series were young adults, but were fed daily except for the short period preceding their autopsy.

These data are not in accord with the observations by Marenzi and Gerschman, who found a marked increase in the blood phosphate of dogs after similar treatment (2, 3).

Discussion. The evidence available at present shows that anterior hypophyseal extracts affect the serum-calcium level through the medium of the parathyroid glands. Such physiological data are of distinct value in the further study of the clinical pathology of pituitary dysfunction. Erdheim, Cushing and Davidoff (16) and others have reported the occurrence of parathyroid hyperplasia or adenomata in acromegaly. Cushing has also found parathyroid adenomata in a patient with basophilic adenoma of the anterior hypophysis (17). Bauer and Aub (18), who recently studied the total calcium metabolism of acromegalic patients, demonstrated that active cases showed a distinctly elevated calcium excretion in the urine, but a normal calcium and phosphorus excretion in the feces and a normal concentration of these elements in the blood.

SUMMARY

Each of 19 guinea pigs was injected intraperitoneally daily for 9 days with 1 cc. of an alkaline extract of the anterior hypophysis. Fifteen guinea pigs on an identical diet were reserved for controls. The extract-treated animals showed a significantly elevated serum-calcium level as compared with the untreated controls. The serum phosphates were not affected by these injections. These data, in addition to other available evidence, indicate that the anterior hypophyseal extract exhibited a parathyreotropic activity.

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A COMPARISON OF SODIUM, CHLORIDE AND CARBOHYDRATE CHANGES IN ADRENAL INSUFFICIENCY AND OTHER EXPERIMENTAL CONDITIONS[1]

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The decrease in plasma sodium and chloride levels and correlated derangements in salt distribution and excretion are considered by some investigators to constitute the crucial phenomena of the syndrome of adrenal insufficiency (see Harrop, 1936). It is well known, however, that experimental surgical procedures other than adrenal excision eventually result in lowered chloride levels in the blood or plasma. Such changes have been found to occur after bilateral nephrectomy (Swingle, 1928) or ureteral ligation (Atchley and Benedict, 1927), and after extirpation of the pancreas (Ni, 1926). Attempts have been made by earlier workers on experimental diabetes to relate the blood chloride with the blood sugar changes (Ni, 1926). In the case of adrenal insufficiency also efforts have been made to find a common factor in the two important failures of blood glucose and sodium chloride maintenance. It was considered possible that an investigation of the effects of various surgical and other procedures might yield results which would indicate relationships between carbohydrate, sodium chloride and water concentrations in adrenal insufficiency.

RESULTS. *Pancreatectomy.* In the later stages of experimental diabetes (in the cat) there was a remarkable lowering of the serum sodium and chloride levels. Serum chloride decreased proportionately more than serum sodium, undoubtedly due to replacement of inorganic anions by the organic acids of diabetic acidosis. Serum sugar reached the average level of 0.6 per cent. Two observations are of interest in this connection—the blood sugar rises to a high level (over 400 mgm. per cent) before the salt content tends to diminish, and the serum sodium chloride levels of severe diabetes are more depressed than those of adrenal insufficiency. Results are given in table 1.

Pancreatectomy plus adrenalectomy. When both adrenal glands and the

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

[2] E. R. Squibb and Sons Scientific Fellow in Physiology.

TABLE 1

Sodium, chloride, water and carbohydrate levels in various experimental conditions

	SURVIVAL	SERUM SODIUM	SERUM CHLORIDES	BLOOD WATER	MUSCLE WATER	SERUM SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN
	days	*m.e./liter*	*m.e./liter*	*per cent*	*per cent*	*mgm. per cent*	*per cent*	*per cent*
Normal* (13 cases):								
Maximum		161.1	128.8	86.8	77.2	100	1.57	0.54
Minimum		151.2	120.5	82.8	74.5	73	0.67	0.34
Average		155.6	123.4	84.9	75.7	87	1.22	0.43
Adrenalectomized* (9 cases):								
Maximum	14	141.0	120.2	82.5	78.7	63	0.11	0.27
Minimum	4	116.0	95.7	77.1	76.0	50	0.04	0.14
Average	8.7	133.0	109.2	80.3	77.4	57	0.07	0.21
Adrenalectomized—pancreatectomized (6 cases):								
Maximum	1.2	175.0	129.9	82.0	77.8	112	0.17	0.67
Minimum	0.5	127.8	114.4	77.6	74.7	54	0.09	0.29
Average	1.0	153.9	120.5	79.9	75.8	73	0.15	0.48
Adrenalectomized-nephrectomized (8 cases):								
Maximum	3	161.1	131.0		78.3	74	0.17	0.46
Minimum	1	143.2	107.0		74.6	49	0.10	0.25
Average	2.3	150.0	113.5		76.9	60	0.13	0.34
Pancreatectomized (5 cases):								
Maximum	7	143.0	110.5	85.2	77.9	800	0.36	0.35
Minimum	3	130.3	66.0	80.9	73.5	398	0.16	0.27
Average	4.2	134.7	91.5	83.0	75.5	597	0.25	0.31
Nephrectomized (9 cases):								
Maximum	4	148.0	111.4		78.6	213	1.02	0.79
Minimum	2	127.8	97.7		75.4	118	0.31	0.28
Average	2.8	138.8	105.0		77.4	131	0.38	0.56
Nephrectomized (no food or water) (7 cases):								
Maximum	4	157.8	119.1	86.1	80.0	172	1.01	0.69
Minimum	1.5	142.0	107.0	78.9	76.0	102	0.11	0.28
Average	3	151.2	111.5	83.6	77.4	136	0.49	0.40
Nephrectomized-pancreatectomized (7 cases):								
Maximum	2	139.5	96.8	85.4	80.8	816	0.28	0.53
Minimum	1	125.0	72.4	83.6	72.8	476	0.18	0.16
Average	1.7	132.0	84.8	84.5	76.0	659	0.21	0.37
Splenectomy (3 cases):								
Average	Indefinite	153.5	121.1			82		
Partial hepatectomy (5 cases):								
Average	2	157.2	126.3	81.9	75.7	129	0.12†	0.11
Intraperitoneal tissue transplants (9 cases):								
Average	3.7	144.7	111.6	81.1	76.0	144	0.30	0.30

* From Silvette and Britton, 1935.

† Amount in remainder of liver.

pancreas were removed in one operation, death resulted in about 24 hours. Animals utilized when showing symptoms were found normal in regard to serum sodium chloride and muscle water and glycogen levels. Serum sugar was significantly reduced, however, and liver glycogen almost completely disappeared. In two cats which were adrenalectomized 7 days after pancreatectomy, the blood sugar (previously varying between 200 and 300 mgm. in simple experimental diabetes) fell 50 per cent within 15 hours.

Bilateral nephrectomy. In agreement with earlier results it was found that bilateral nephrectomy resulted in a decrease in serum sodium and chloride. One series of tissue analyses showed that muscle and liver, at least, did not gain salt at the expense of the blood. Thus the fate of sodium chloride lost from the blood is not apparent from these experiments. That it is in part due to dilution of body tissues appears from a consideration of the muscle water content of nephrectomized animals. No vomiting was observed.

The matter was further investigated by withholding both food and water in a group of nephrectomized cats. In this series survival was somewhat prolonged, but at the time of sampling it was found that serum sodium and chloride levels, while below normal, were considerably higher than those of nephrectomized animals allowed water *ad lib.* The muscles were abnormally hydrated. In both series, serum sugar was increased well above the normal, and liver glycogen was notably but not critically reduced.

Nephrectomy plus adrenalectomy. Serum sodium and chloride and blood water levels were somewhat reduced following removal of both kidneys and adrenals in a one-stage operation. Serum sugar and liver and muscle glycogen were, however, seriously affected, reductions being noted to approximately the levels observed in uncomplicated adrenal insufficiency.

Nephrectomy plus pancreatectomy. Removal of both kidneys and the pancreas resulted in an increase in serum sugar above that noted in simple pancreatectomy. The excess sugar also forced an additional amount of sodium chloride out of the blood plasma. In these experiments, indeed, serum sodium chloride levels were found to be lower than in any other operative condition investigated (see, however, effects of glucose injection, fig. 1, A).

Various abdominal operations. Splenectomy produced no effect on the apparent normal health of cats, and serum sodium and chloride and sugar levels were unaffected. Removal of a large part of the liver resulted in very slight increases in serum sodium chloride and blood concentration, and the water content of the muscles was unchanged. The insertion of several small portions of fresh tissue (liver, kidney, spleen, pancreas or adrenal) free in the abdominal cavity, which usually resulted in death

within a few days, had variable effects on salt levels in the plasma; in general, reductions were noted.

Discussion. Serum sodium and chloride levels have been observed to suffer reduction not only following removal of the adrenal glands, but after pancreatectomy, nephrectomy and many other abdominal operations. The mechanism of sodium chloride loss in adrenal insufficiency must perhaps be considered on a different basis from that involved in pancreatectomy and nephrectomy. Thus it may be significant that the ratio of sodium to chloride loss from the serum is 1.26 in cases of adrenal insufficiency, while in all other experimental conditions investigated the ratio averages 0.59 (range, 0.45 to 0.74—see table 2). In the normal unoperated cat the ratio of serum sodium to chloride is found also to be

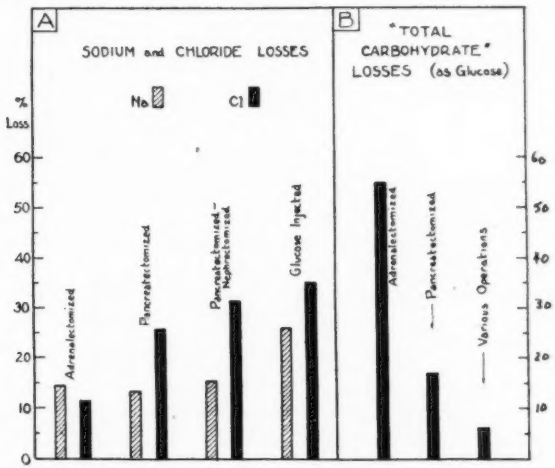

Fig. 1. Effects of various operative procedures on blood and tissues (cats).

1.26. Following injection into the normal animal of hypertonic saline solution, however, the increases in serum sodium and chloride show a ratio of 1.02.

Evidence has been presented showing that animals may succumb to adrenalectomy with the serum sodium and chloride levels either increased or unchanged (Silvette and Britton, 1936) or decreased. Different results are observed in different mammalian types. When decreases occur they are a function of the survival time—the longer the life-span after operation the greater the amount of salt eliminated by the kidneys and the lower the ultimate level in the blood. Furthermore, much greater sodium and chloride reductions are observed after glucose injection, and in many operative conditions also the chlorides are more seriously de-

pressed. Considered on the basis of change per day, the rate of fall of ions in the blood is found to be from 5 to 15 times as great in the different (extra-adrenal) operations considered, compared to cases of adrenal insufficiency. Diminutions in serum sodium and chloride may be said to be of normal occurrence after abdominal operations.

In general the water content of skeletal muscle varies inversely as the serum sodium chloride level, or more explicitly, with the serum chloride concentration. Hence, most operations which are sufficiently serious to bring the animal to the point of death result in increases in muscle water percentage and decreases in serum sodium chloride concentration. It is questionable, however, whether the salt depletions in the present

TABLE 2

Comparison of sodium chloride and carbohydrate changes in adrenal insufficiency and other conditions

	PER CENT LOSS IN SERUM Na AND Cl		RATIO Na LOSS TO Cl LOSS	PER CENT CHANGE FROM NORMAL CARBOHYDRATE CONTENT			
	Na	Cl		Serum sugar	Liver glycogen	Muscle glycogen	"Total" CHO†
Adrenalectomized*	14.5	11.5	1.26	−34	−94	−51	−55
Adrenalectomized-pancreatectomized	1.1	2.4	0.46	−16	−88	+11	0
Adrenalectomized-nephrectomized	3.6	8.0	0.45	−31	−90	−21	−29
Pancreatectomized	13.4	25.8	0.52	+586	−80	−28	−17
Nephrectomized	10.8	14.9	0.72	+51	−69	+30	+20
Pancreatectomized-nephrectomized	15.2	31.3	0.49	+660	−83	−14	−3
Intraperitoneal tissue transplants	7.0	9.6	0.73	+66	−76	−30	−33
Glucose injected*	26.0	35.0	0.74				

* From Silvette and Britton, 1935.

† Glucose equivalent. Calculated on basis of blood 7 per cent, liver 2 per cent and muscle 45 per cent of body weight.

experiments were in any case (including adrenalectomy) the first or specific cause of death.

Unless the adrenal mechanism were involved, there occurred an augmentation of blood glucose in all the foregoing experiments. Removal of the adrenals appeared to be followed invariably by downward shifts in blood sugar and serious losses in carbohydrate (blood sugar plus liver and muscle glycogen) in the body.[3] In no other operative condition

[3] Various observations from this and other laboratories indicate that skeletal muscle glycogen, liver glycogen and blood glucose account for approximately 85 per cent of the total carbohydrates in the body. Glycogen (in smooth muscle, heart, etc.) and free sugar in other tissues make up under normal conditions probably no more than 15 per cent of the body carbohydrates.

which we have investigated does the carbohydrate content of the body fall nearly as low as in adrenal insufficiency. Hepatectomized animals only would appear, from the work of others, to suffer greater losses (Bollman, Mann and Magath, 1925). In experimental diabetes—which involves an acknowledged critical disturbance in carbohydrate metabolism—less than one-third the amount of carbohydrate (17 per cent compared with 55 per cent in adrenalectomy) appears to be lost (fig. 1,B). Furthermore, animals dying of adrenal insufficiency on either the second or the eighth day after operation manifest similar critically low blood sugar levels (Britton and Silvette, 1932). This is in striking and significant contrast to serum sodium and chloride changes, which are non-critical and occur in proportion to survival time.

SUMMARY

A number of different abdominal operations (cats) result in decreases in serum sodium and chloride. In some cases reductions two and three times as great as those found in adrenal insufficiency have been observed. Calculated on the basis of rate of change per day, the salt losses after adrenalectomy are relatively small and non-critical.

It is possibly significant that the ratio of serum sodium to serum chloride *loss* in adrenal insufficiency is 1.26 in comparison with ratios between 0.4 and 0.7 (average, 0.59) in other conditions investigated—pancreatectomy, nephrectomy and various combined operations. It is shown that the normal serum Na/Cl ratio in the cat is 1.26, i.e., similar to the ratio of the losses in adrenal insufficiency. In the normal animal injected with saline solution, *gains* in serum sodium and chloride show a ratio of 1.02.

In all the experiments studied, except adrenalectomy, there were observed increases in serum sugar following operation. Removal of the adrenal glands, whether alone or in conjunction with other operations (e.g., adrenalectomy *plus* pancreatectomy) resulted in reductions in serum sugar levels. More than one-half (55 per cent) of the carbohydrate content of the body (liver and muscle glycogen and serum sugar) disappeared after adrenal removal in comparison with 17 per cent loss in pancreatectomized animals.

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THE EFFECT OF CHANGES IN pH ON THE ACTION OF MAMMALIAN A NERVE FIBERS

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The purpose of the experiments described in this paper is to ascertain the conditions which will permit isolated mammalian nerve to yield action potentials of the same form as those that must obtain during physiological functioning in the body.

As seen when mounted in an incubator in an oxygen atmosphere, the nerves display after-potentials of very variable form; and as has been described by Adrian, they are often the seat of spontaneous discharges. Both these phenomena suggest strongly that the activity has been modified by an alteration of the medium in which the nerve is placed. Changes of pH, to which nerves are known to be sensitive (Adrian, 1920; Davis, Pascual and Rice, 1928; Necheles and Gerard, 1929; Gasser and Erlanger, 1930), come first to mind, for the gas to which the nerve is exposed has been changed without altering its salt content. Also, there is the possibility that the salts in Ringer's solution are not sufficiently exact duplicates of the salts in the blood, and that some of the organic constituents of plasma are necessary for proper functioning.

In accord with these considerations, experiments have been carried out with both blood and Ringer's solution and with buffered physiological salines prepared with different hydrogen ion concentrations. The laws of the variation of the action potential with the experimental conditions have been defined and the conditions which give a normal potential identified. The basis for the selection of the latter is the excitability curve. Excitability changes as the after-potentials change, and it is argued that those after-potentials must be normal which occur in nerves that yield excitability curves having the same contour as those found in the body (Gasser and Grundfest, 1936).

METHODS. 1. *Nerve chamber.* A small water-tight nerve chamber which could be immersed in a water bath was employed. The electrodes were arranged so that by a turn of the chamber, the nerve could be either immersed in the physiological saline or exposed to the gas mixture (fig. 2). The nerves were dissected under warm saline and immediately transferred

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to the warm chamber which was also filled with saline during the mounting. Nerves not intended for immediate use were kept at 37°C. in buffered saline equilibrated with O_2 and CO_2 to a pH of 7.4.

2. *Physiological saline.* As pointed out by Krebs and Henseleit (1932), the usual composition of Ringer's solution does not agree with the electrolyte content of mammalian blood sera, especially with respect to PO_4, Mg, SO_4, and in some cases with respect to K. As the excitability of the nerve is strongly influenced by the ratio Ca^{++} to K^+, this point seems to be of interest in experiments with surviving nerve. The magnesium ion should

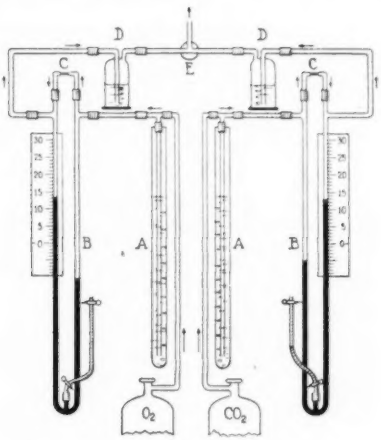

Fig. 1. Diagram of gas flowmeters. The gas first passes the safety valve (A) which protects against sudden rises in pressure that might force the liquid (colored water) in the flowmeter (B) out of the U-tube. Having passed the flowmeter capillaries (C), the gas bubbles through a 250 cc. wash-bottle (D) half filled with mineral oil which acts as a gas pressure regulator. The gases (O_2 and CO_2) are mixed in the mixer (E), a T-tube with a three-way stopcock. The scale readings are graduated in centimeters. Length of A and B = 1 meter. Diameter of B = 8 mm. For further information, see text.

also receive more attention than has generally been accorded to it, inasmuch as Fenn and co-workers (1934) have shown that nerves have a relatively high content of Mg. In the solution used by Krebs the content of the various ions is in agreement with the values commonly found in mammalian blood sera.

Krebs' solution, with 0.1 per cent glucose, will be referred to in the following text as "complete Krebs' solution." For some purposes the solution is used without KH_2PO_4 and $MgSO_4$, and it is then termed "incomplete Krebs' solution." In these instances the omitted salts are replaced by an osmotically equivalent amount of NaCl.

3. *Gas mixtures and pH measurements.* The pH was controlled by equilibrating the fluids in the chamber with O_2 containing different partial pressures of CO_2. The content of CO_2 was determined by a system of flowmeters (fig. 1) adapted from the type which has been used by Michaelis and Flexner (1928). Optimum operation was obtained when the gases from the high pressure cylinders were adjusted to about 15 pounds of pressure by means of the reducing valves, and the rate of flow was determined by needle valves inserted between the reducing valves and the flowmeters. Mixtures containing from 2.5 to 5 per cent of CO_2 could be supplied at rates of flow ranging between 200 and 1000 cc. per minute; 7.5 per cent of CO_2 could be supplied in the range between 200 and 600 cc. per minute, and 10 per cent in the range between 200 and 400 cc.

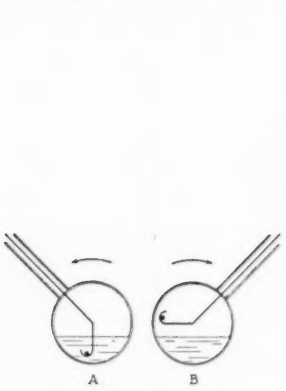

Fig. 2

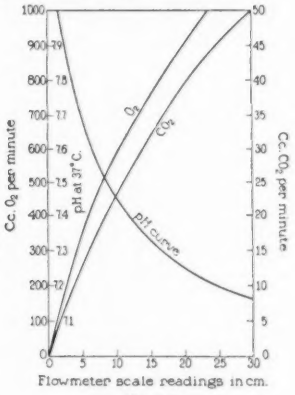

Fig. 3

Fig. 2. Cross section of the nerve chamber with the nerve dipped in the Krebs solution (A) and raised for recording of potentials (B).

Fig. 3. Calibration diagram for the flowmeters showing the output volume per minute of the gases as given by the flowmeter scale. The "pH curve" presents the pH values in the bicarbonate buffered Krebs solution when the oxygen output was fixed at 600 cc. (10 cm. scale reading) and the CO_2 output was varied from 2.5 to 30 cm., scale reading.

When the volume per minute did not need to be altered, it was convenient to maintain the oxygen outflow constant and to vary the CO_2. In most of the experiments 600 to 700 cc. per minute were used. Oxygen in the amount of 600 cc. per minute was delivered at a flowmeter reading of 10 cm., and the CO_2 content was varied so as to give pH values between 7.1 and 8.1 by changing the reading of the CO_2 flowmeter between 30 cm. and 0. The relation between the rate of flow of CO_2 and the pH of the solution is shown in figure 3.

Calibration of the flowmeters can be accomplished by analysis of the gas mixtures (and the pH of the solution then calculated from the Hassel-

balch-Henderson equation (Peters and van Slyke, 1931)), or by measurement of the pH of the equilibrated solutions. The latter method was the one chiefly used, but checks were made to prove that the two methods were in agreement.[2]

A sample of the gas-saturated Ringer's solution was collected under paraffin, and the pH determined at 30°C. with the glass electrode. Under these conditions, the change in pH from that determined at 37°C. is dependent only upon the temperature coefficient of pK_1 of carbonic acid, and according to the measurements of Shedlovsky and MacInnes (1935), this is very small and has been negligible for the difference of 7°C. The accuracy of the pH determination was ±0.015 pH. The pH in the Krebs solution varied during the experiments within a range of ± 0.03 pH from the desired value as the result of small irregularities in the gas flow, occasioned in part by the flowmeters and in part by condensation of water in the output tubing from the nerve-box. In changing from one pH to another with an output volume of the gas mixture of 600 cc. per minute, 20 cc. of solution attained equilibrium within from 5 to 6 minutes after starting to bubble the gas through the solution. The gas mixture was saturated with moisture at 37°C. before entering the nerve chamber.

4. *Stimulating and recording apparatus.* The nerve was stimulated by break shocks from a Porter coil or by thyratron discharges, and the potentials were recorded monophasically with the cathode ray oscillograph, using a D.C.-amplifier for the study of the after-potentials, and an A.C.-amplifier for the spikes. For further information regarding the apparatus, see Gasser and Grundfest (1936).

RESULTS. *Comparison of the activity of the phrenic nerve in defibrinated blood and in Ringer's solution.* In the first experiments, which were carried out on the phrenic nerve of the cat, it was observed that the spontaneous discharge which occurred regularly in nerves bathed in unbuffered Ringer's solution could be stopped by the application of blood equilibrated with 5 per cent of CO_2 in O_2, an observation which had previously been made by Adrian (1930). The threshold for electrical stimulation rose when the nerves were moistened with blood, and the after-potentials changed in a manner recalling the effect of application of CO_2 (Davis, Pascual and Rice, 1928). It seemed possible, therefore, that the effect of blood was attributable to the reaction obtained.

When arterial blood taken from the narcotized (ether) animal was examined with a glass electrode, samples of defibrinated blood saturated with 5 per cent of CO_2 in O_2 and samples of heparinized blood collected under oil were found to have pH values ranging between 7.2 and 6.9,

[2] The author is indebted to Doctor L. Michaelis for his kindness in permitting the use of his apparatus for pH determinations.

measured at 30°C. A lactic acid content 50 to 100 per cent above normal accounted in part for the acidosis.[3]

These findings indicated that the changed behavior of the nerve in blood could indeed be attributed to an acid reaction; but they also showed that blood taken from the animals which supplied the nerves was abnormal on account of an outpouring of fixed acid during the preparatory operations. Further experiments revealed that the obtaining of completely normal blood was a very difficult procedure, and hence attention was turned to buffered salines in order to learn to what extent the activity of nerve could be maintained in a normal state through control of the pH alone.

TABLE 1

Spike potential data obtained at various pH values from single nerve fiber preparations, spinal root, of the cat

The percentage decrease of the spike height at pH 7.7 is calculated in relation to the height at pH 7.4, and the percentage increase in conduction rate at pH 7.7 in relation to the conduction rate at 7.4.

EXPERIMENT NUMBER	pH	TEMPERATURE	SPIKE DURATION, MSEC.	RISING PHASE		SHOCK-SPIKE TIME		NUMBER OF PICTURES
				In msec.	In per cent of spike duration	Duration, msec.	Per cent decrease at pH 7.7	
1	7.4	36.8	0.43	0.16	37.3	0.36		30
2	7.2	37.0	0.38	0.143	37.6	0.26		6
	7.4	37.0	0.36	0.130	35.2	0.30		23
	7.7	37.0	0.35	0.143	41.0	0.26	16	11
3	7.4	37.0	0.365	0.135	37.0	0.311		9
4	7.4	36.5	0.525	0.173	33.0	0.45		9
	7.7	36.5	0.485	0.180	37.1	0.385	16	12
5	7.4	37.5	0.493	0.183	38.1	0.332		12
6	7.2	37.5	0.450	0.180	40.0	0.405		6
	7.7	37.5	0.435	0.167	38.2	0.35	15.8	8
7	7.2	38.0	0.50	0.190	38.0	0.448		6
	(7.7)	(11)	(0.43)	(0.225)	(52)	(0.415)	(13)	1
8	7.2	38.0	0.385	0.142	38.0	0.415		12
	7.7	38.0	0.384	0.153	40.0	0.370	(13)	7
9	7.2	38.0	0.45	0.164	36.4	0.384		6

The earliest experiments sufficed to show that the behavior of a nerve mounted in oxygen, as opposed to the behavior of the same nerve treated with serum, could be imitated in a nerve mounted in buffered Krebs' solution simply by altering the pH. When the pH was shifted to the alkaline side, the potential manifestations were like those in the former case; when it was shifted to the acid side, the manifestations were like those in the

[3] The author is indebted to Doctor E. Shorr for the lactic acid determinations which were made in his laboratory.

latter. As the result of this finding a study of the effect of pH in various aspects of nerve activity was started. In these experiments, incomplete Krebs' solution was used because fewer changes in the ionic content are involved as the pH is changed, when $MgSO_4$ and KH_2PO_4 are absent.

The effect of a change in pH of the Krebs solution on the action potential. The spike potential. The persistence of the form of the spike potential in the face of changes in experimental conditions is well known for frog nerve, and seems to be characteristic also for the spike of mammalian A fibers. Nevertheless, extreme changes in pH have shown that certain reversible changes can be produced. The observations were made on single fiber responses in strands of spinal roots and the data are presented

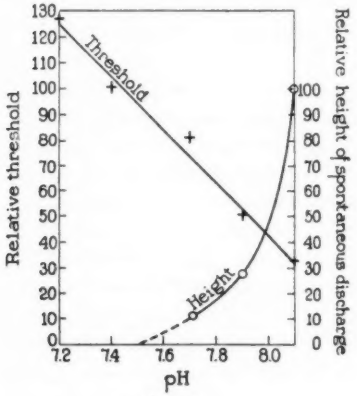

Fig. 4

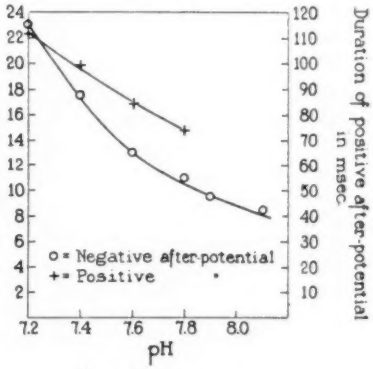

Fig. 6

Fig. 4. Relation between relative threshold for electrical stimulation and pH in a phrenic nerve. The threshold at pH 7.4 is called 100. The "height" curve represents the medium height of the spontaneous discharge at various pH values; the height at pH 8.1 is called 100.

Fig. 6. Duration of negative and positive after-potentials in the phrenic nerve (cat) at various pH values (April series).

in table 1. The mean value of the spike duration at 37° to 38°C. and pH 7.2 to 7.4 in the experiments described in table 1, and in other experiments which will be published in another connection, was 0.43 msec., with 0.36 and 0.50 as extreme values. These durations are in good agreement with those given by Gasser and Grundfest for 37.5°C., namely, 0.40 to 0.43 msec. The rising phase occupies 37 to 38 per cent of the spike duration.

When the pH is increased from 7.2 or 7.4 to 7.7, there seems to be a shortening of the spike duration. In order to diminish the errors in determining the exact point of the starting and ending of the spike, each value given was based on the analysis of a large number of pictures (table 1). While the change in duration falls almost within the limit of

error, it is always in one direction; so the shortening observed may be real. It may possibly be secondary to the small increase in conduction velocity. The ratio of the rising phase to the total duration was not affected. When the pH was higher than 7.7, irreversible changes appeared at times, and therefore the higher alkalinities were not systematically investigated.

The height of the spike as studied in the enlarged picture showed in all cases a reversible decrease at the higher pH values.

Conduction rate. The shock-spike interval in root preparations was 13 to 16 per cent shorter at pH 7.7 than at pH 7.4 or 7.2. As this interval depends partly on conduction rate and partly on utilization time, observations were also made on the phrenic nerve in which longer conduction distances could be employed. In 5 of 9 experiments the conduction rate was unchanged at the higher pH, and in 4 experiments it was increased by about 15 per cent.

The spike threshold and its relation to spontaneous discharge. The spike threshold for electrical stimulation was affected in a most striking manner by a change in pH, as seen from the data shown in figure 4. The intensity of the threshold stimulus at pH 7.4 was called 100, and the thresholds at other pH values were calculated in relation to this value. The figure shows that there was a linear relationship between a change in pH and the corresponding change in the relative spike threshold. For a difference of 0.1 pH there was a change of about 10 units in the relative threshold. At pH values higher than 7.8 there was, in some preparations, a deviation from a straight line, indicating an acceleration in the rate of drop in threshold; but too much attention should not be paid to this deviation, as the CO_2-bicarbonate buffer system has very little buffering capacity at these pH values.

A change in the above direction in the threshold for electrical stimulation of frog nerve with changes in reaction was observed by Adrian (1920). But little attention seems to have been paid to the physiological importance of such changes. Reaction, threshold, and the amount of spontaneous discharge are closely linked together. As the threshold is reduced by a progressive increase in alkalinity, the amount of discharge increases regularly in relation to it. At the reaction of its first appearance (pH 7.6 to 7.7) the discharge is seen only during the supernormal periods following a maximum spike. In more alkaline nerves, on the other hand, the discharge is spontaneous and continuous. As the pH is increased, the frequency and height of the components of the discharge become larger. Figure 4 shows the mean heights of the components at the various pH values, plotted in terms of the height at the highest pH listed.

All the phenomena which have been described in connection with shifts in pH are completely reversible. Restoration of an alkaline nerve to a normal reaction is attended by a rise of threshold and cessation of dis-

charge; and alternation between normal reaction and alkalinity may be carried out repeatedly with the recurrence of the same events.

The pH value at which the spontaneous discharge was first visible—the "critical pH value"—varied within certain limits, indicating the existence of some other variable (presumably the ionic content) affecting the excitability of the nerve. When complete Krebs' solution was used, the "critical pH value" was about 0.1 pH to 0.15 pH higher than when incomplete Krebs' solution was employed. This difference, it will be pointed out in a succeeding paper, can be explained on the basis of the presence or absence of magnesium.

After-potentials. The variations of the after-potentials described by previous observers are stages in a continuous series which can be controlled by the reaction of the solution and reproduced reversibly at will. To pass from the non-rhythmic to the rhythmic potentials described by Gasser and Grundfest (1936), it is necessary only to change from a normal to a more alkaline reaction.

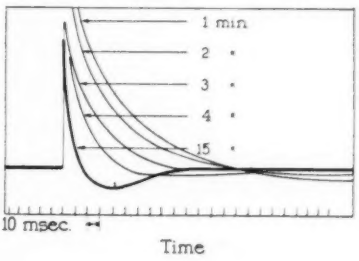

Fig. 7. Successive stages in negative and positive after-potentials in the phrenic nerve at various time intervals after a rapid change in pH from 8.1 to 7.4. The heavy line represents the shape of the after-potentials at pH 7.4 before the rise to 8.1. (The shape of the after-potential at pH 8.1 is not given in the figure, but is similar to that shown in fig. 5.) The increase in the negative after-potential has reached its maximum after 1 minute and decreases thereafter to the normal shape at pH 7.4, which is attained after 15 minutes.

A series of characteristic pictures of the after-potentials at different pH values is shown in figure 5. The general form of the after-potentials is described by Gasser and Grundfest (1936) as consisting of a negative potential starting before the decline of the spike, followed by a more or less marked positive potential of longer duration. Only the progressive changes, with shifts in pH, need be mentioned here. It will readily be seen that the main changes produced by an increase in pH consist of a shortening in duration of both potentials in a way so that they are moved up closer to the spike. The positive after-potential is increased in depth and area, while the area of the negative potential is decreased. The relation between the duration of the first negative and positive potentials to pH is shown in figure 6.

Parallel with these events, a second negative after-potential develops. As a rule it is clearly visible at pH 7.7, and at higher pH values it is often followed by additional positive and negative waves. Even at physiological pH values, however, there are sometimes signs of the second negative

potential, and in one preparation two secondary negative potentials were seen. If still higher amplification had been used, these waves possibly would have been traced more frequently at normal pH values. There is no doubt a regular transition throughout the range from low, long-period waves at normal reaction to the high, short-period waves in the alkaline condition.

The time required for the stabilization of the potentials after a shift in pH was about ten to fifteen minutes for the phrenic nerve, five minutes of which were accounted for by the time required for the pH in the Krebs' solution to reach equilibrium. When equilibration was achieved, the potential picture was stable and the height of the spontaneous discharge remained constant. At the highest pH values observed, progressive changes of a more or less irreversible nature were seen. All the potentials became smaller and the excitability of the nerve gradually decreased.

When the pH was shifted from a higher to a lower value, the reversibility of the potentials was clearly demonstrated, but the potentials did not immediately change to the picture characteristic of the new pH value, as they did for a pH shift in the opposite direction. Intermediate stages with a very large negative potential and a small positive potential occurred, and the normal configuration was not reached until from ten to fifteen minutes had elapsed. Figure 7 shows an example of these events in experiments on the phrenic nerve. In the saphenous nerve the same phenomena were demonstrated, but here the equilibrium was not reached until after thirty to forty minutes. Figure 8 shows the negative after-potential at the time of its greatest magnitude in this nerve, twenty-eight minutes after the solution was changed to pH 7.4.

Nerves vary in their behavior, both with respect to the amount of spontaneous discharge at the various reactions and with respect to the magnitude of the oscillations in the rhythmic after-potential. The sciatic nerve behaves like the phrenic nerve, but in the saphenous nerve these phenomena are much less marked (fig. 8). In the latter nerve, spontaneous discharge first begins at pH 7.9–pH 8.0, and secondary waves of negative and positive after-potentials are rarely seen. When the nerve is made alkaline, decrease of the first negative after-potential and increase in the positive after-potential occur, however, in exactly the same manner as they do in the other nerves.

The experiments which have been described were performed during the months of January to May, inclusive. Similar experiments made during the months of September and October showed that nerves under comparable conditions were less susceptible to spontaneous discharge, and had a less marked rhythm in the after-potentials.

Excitability curves. Curves of excitability were mapped by the method of thresholds at two pH values, 7.4 and 8.0, and close correspondence was

found between them and the contours of the after-potentials under the same conditions (fig. 9). In passing from pH 7.4 to pH 8.0 the period of supernormality was shortened, and the following period of subnormality was both shortened and intensified. The subnormality was then succeeded by supernormality and the curve continued in an oscillating form paralleling the potential. Such deviations from exact parallelism as appear can be accounted for by the fact that the form of the potential is changed somewhat by the process of measuring the thresholds and by the fact that the potential picture for all the fibers may not be completely representative of the threshold fibers.

The point of greatest interest which emerges from these observations is that maintenance of the nerve at pH 7.4 supplies the necessary conditions

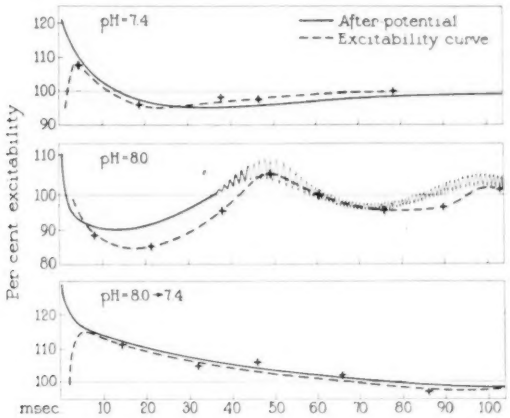

Fig. 9. Relation between excitability curves and after-potentials in a phrenic nerve (cat) at pH 7.4 and 8.0, and during a shift from pH 8.0 to 7.4. Spontaneous discharge in the after-potential at pH 8.0 is schematically drawn.

for the recording of the normal after-potential form. The proof of this statement lies in the fact that the durations of the parts of the excitability cycle coincide with the durations found in the body. In the series of 9 experiments the relatively refractory period was found to end after 2 to 5 msec.; the supernormal period ended after 10 to 20 msec., and had a maximum between 4 and 7 msec.; and the subnormal period ended after 60 to 90 msec., and had a rather flat maximum between 30 and 50 msec. Gasser and Grundfest found for nerves in the body a relatively refractory period lasting from 2.5 to 5 msec., a supernormal period ending after 12 to 18 msec., and a subnormal period ending after 60 to 80 msec. In view of the close correspondence between excitability and the after-potentials, not only in the present experiments but in many previous experiments, there

is good reason to believe that the potentials here recorded at pH 7.4 ar representative of those obtained in the body. The correspondence is als supported by a comparison of the magnitudes of the deviations in excit ability in the two cases. Maximum supernormality of 2 to 30 per cen (average 9 per cent) in the present series is to be compared with 0 to 20

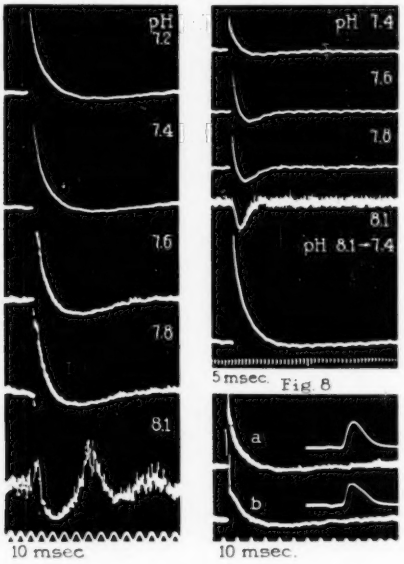

Fig. 5 Fig. 10

Fig. 5. Successive stages in the changes in negative and positive after-potentials in the phrenic nerve after a single maximal shock, when the pH is changed from 7.4 to 8.1. Temperature 37°C. Conduction distance, 5 cm. Distance between the leads, 2.5 cm.

Fig. 8. Successive changes in the after-potentials of the saphenous nerve (cat) following an increase in pH. The maximum increase in negative after-potential was recorded 28 minutes after the pH was changed from 8.1 to 7.4 and is shown in the last picture. Temperature 37°C. Conduction distance, 5 cm., and lead distance, 2.5 cm.

Fig. 10. Maximum spike and after-potentials in a phrenic nerve—(a) fresh, (b) after 26 hours at 37°C. and pH 7.4. Conduction distance, 5 cm. Lead distance 2.5 cm.

per cent (average 7 per cent) in Gasser and Grundfest's series; and similarly, maximum subnormality of 2 to 4 per cent is to be compared with 0 to 6 per cent.

The lifetime of the nerve. Phrenic nerves can usually be kept in fairly good condition for 10 to 20 hours at pH 7.4 and 37°C. Figure 10 shows comparative records of the spike and after-potentials in a fresh nerve

(fig. 10 a), and in the same nerve 26 hours later (fig. 10 b). The spikes are alike, but the after-potentials in the kept nerve are somewhat smaller.

DISCUSSION. The great sensitivity of the after-potentials to the reaction of the medium about the nerve, which has been described in the foregoing experiments, emphasizes the necessity of precise control of the pH if constant and reproducible responses are to be obtained from the nerve. It is no less imperative to maintain the pH of the nerve with the aid of buffer salts of physiological composition in equilibrium with a normal partial pressure of CO_2, than it is to control the temperature or furnish an adequate supply of oxygen.

Evidence is given in the paper that isolated nerves in a properly controlled environment behave similarly to nerves in their physiological state in the body. A corollary of this observation would be that the more alkaline nerves resemble nerves in states of clinical tetany, such as might result from hyperventilation or other conditions reducing the activity of the calcium.

The present experiments provide information bearing on the interpretation of the nature of the process which is responsible for rhythmicity in the potential. Gasser and Grundfest in their description of the rhythm argued that it is a primary phenomenon in the after-potential, and that it is not secondary to the spontaneous discharge which usually accompanies it, the variation in the intensity of the discharge being the result of the variation of excitability which accompanies the oscillation of the after-potential, rather than the cause of the rhythm. This view is now supported by the observation that waves in the after-potential can be clearly traced in the phrenic nerve at normal pH values when there are no signs of spontaneous discharge. The large waves seen at high pH values (pH 8.1 in fig. 5) bring out most strikingly that cyclic phenomena occur in peripheral nerve, as in the central nervous system, and are capable of yielding rhythmic potentials with long periods.

SUMMARY

A method for rapidly changing the hydrogen ion concentration of the medium about an isolated nerve is described, and the effects of changes in pH on the responses of mammalian A fibers are reported.

The striking effects of pH changes are seen on the threshold of excitation and the behavior of the after-potentials. The spikes are but little affected.

The threshold for electrical excitation with induction shocks is increased when the nerve becomes acid, and decreased when the nerve becomes alkaline, the variations being nearly linear with the pH in the range of 7.2 to 7.8. The change is reversible.

When the threshold falls in alkaline nerves, spontaneous discharge develops. The latter usually becomes visible at pH 7.6 to 7.7 in the

phrenic nerve, and increases in height and frequency as the pH is further increased. Restoration of normal pH promptly stops the discharge.

The after-potential at pH 8 is definitely rhythmic. Starting with a negative potential, it oscillates between negative and positive; but it is rapidly attenuated and can be traced for only a few cycles. When the pH is decreased toward normal, the form of the potential changes. The first negative phase increases in magnitude, and the subsequent phase decreases; and at the same time the period of the oscillation becomes longer. Some signs of an oscillation may be visible even at pH 7.4. At normal pH, the first negative after-potential lasts 12 to 20 msec., the positive after-potential lasts 85 to 120 msec., and there may be some trace of a second negative potential.

The excitability curve at various pH values has a contour resembling the potential curves, supernormality corresponding to negativity, and subnormality to positivity. At pH 7.4 there is a close correspondence between the curve obtained and those plotted by Gasser and Grundfest for nerves in the body, indicating that the potentials recorded at this reaction must be normal.

The small changes which were observed in the spikes are described in the text.

The author desires to express his deep appreciation and thanks to Dr. Herbert S. Gasser for his advice and encouragement in this work.

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THE EFFECT OF CHANGES IN THE POTASSIUM-CALCIUM BALANCE ON THE ACTION OF MAMMALIAN A NERVE FIBERS

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In a previous paper (Lehmann, 1937) it was shown that a change in the pH of the saline solution bathing an excised mammalian nerve causes an alteration of the threshold for electrical stimulation, the amount of spontaneous discharge, the form of the after-potentials, and the size of the spike (A fibers). In the present paper data will be presented dealing with the mechanism of these pH effects.

The state of the ionization of the calcium was the first possibility to be considered. If the changes were brought about through alteration in the activity of the calcium ions, the same changes should be producible when the balance between calcium and potassium ions is altered without an accompanying shift of pH; for example, through employment of solutions containing an abnormal ratio of calcium salts to potassium salts, or through deionization of the calcium by the addition of citrate. The following experiments will show that all the phenomena which have been described as occurring when the hydrogen ion concentration is changed can also be produced when the balance between calcium and potassium is varied at constant pH.

EXPERIMENTAL. The methods used were the same as those described in the previous paper (Lehmann, 1937). As the phrenic nerve displays striking changes when the hydrogen ion concentration is altered, this nerve was used exclusively. Krebs solution containing 0.1 per cent glucose but no KH_2PO_4 or $MgSO_4$ was employed. The latter salts were excluded in order to reduce the number of variable factors affecting the nerve.

In some preliminary experiments in which the concentration of the KCl or the $CaCl_2$ in the Krebs solution was increased five to eight times, it was found that on account of the slow exchange of the salts between the fibers and the solution only very slight changes in the action potentials were produced at the end of an hour. The procedure was therefore modified and Krebs solution without $CaCl_2$, or without KCl, as the case might be, was employed. Normal action potentials were first recorded at pH

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values from 7.4 to 8.0, then the potentials in the modified saline at pH 7.4 and finally the potentials in the modified saline when the pH was shifted through the range pH 7.4 to pH 8.0.

Even when the calcium was excluded from the solution the alteration took place slowly. Figure 1 shows the course of events in a nerve in which the changes took place rather more rapidly than they did in the average experiment. After a one-half hour exposure (fig. 1*b*) to the $CaCl_2$-free solution at pH 7.4 spontaneous discharge was visible and the after-potentials were those which a nerve in a physiological salt solution would have at

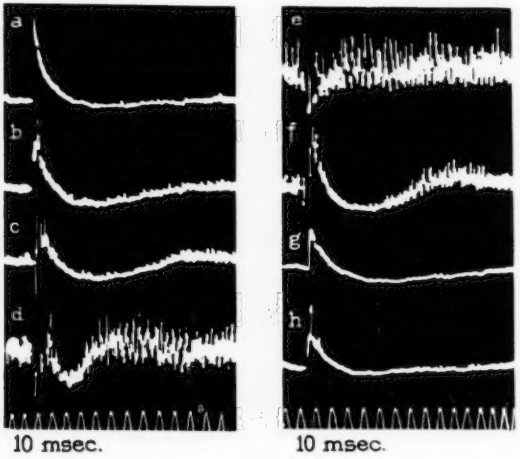

Fig. 1. Changes produced in the after-potentials (phrenic nerve) at constant pH (7.4) by exclusion of calcium from the Krebs solution: *a*, before exclusion of calcium; *b*, 30 minutes; *c*, 1½ hours; *d*, 3½ hours, and *e*, 5 hours after exclusion of calcium; *f*, pH changed to 7.2, and *g*, to 6.9—5 hours after the exclusion of calcium; *h*, after-potentials in the same nerve recorded after *g*, at pH 7.4 following 1 hour's soaking in Krebs solution with normal calcium content but without potassium. Temp. 38°C. Conduction distance, 5 cm. Lead distance, 2.5 cm.

pH 7.7. Progressive increase in the discharge and shortening of the period of the after-potential rhythm then occurred, until after three and one-half hours the potential picture was that which would normally be seen at pH 8.1 (fig. 1*d*). After five hours (fig. 1*e*) the discharge was more violent than one sees in normal nerves at any reaction. At this point, however, a normal picture could still be obtained by ionizing the residual calcium by acidification of the nerve. Ten minutes at pH 7.2 changed the potential picture markedly in the direction of normal (fig. 1*f*), and at pH 6.9 records were obtained which were nearly identical with those obtained initially in normal Krebs solution at pH 7.4 (fig. 1*g*).

As restoration takes place but slowly in a solution with a normal salt content, Krebs solution normal for $CaCl_2$ but without KCl was employed to bring the ion balance back toward normal after it had been upset through depriving the nerve of calcium. After one hour in the latter solution the potential picture at pH 7.4 (fig. 1*h*) showed no signs of spontaneous discharge and a normal form of the after-potential. At this point the nerve had been the subject of experimentation for six hours and had undergone only a very small amount of deterioration.

Absence of calcium, like a decrease in hydrogen ions, caused a striking increase in the excitability of the nerve as measured with induction shocks.

When the KCl content of the solution was increased, the $CaCl_2$ being normal, changes of the same type, though less pronounced, were produced as occurred in a $CaCl_2$-free solution. The effects were the same no matter which method was used for bringing about a given unbalanced calcium-potassium ratio.

In nerves in which the effects of absence of calcium at pH 7.4 manifested themselves more slowly than they did in the nerve described in figure 1, the consequences of the change of ionic balance which had been produced could be made more apparent by examining the nerve at more alkaline reactions. The potential pictures obtained in the nerve at four hydrogen ion concentrations, when the saline composition was normal, are shown in the first column of figure 2. The changes are small as the nerve is one of the October series mentioned in the previous paper (Lehmann, 1937). At each pH value there is shown beside it (column 2) the corresponding picture in the nerve one hour after it had been immersed in a $CaCl_2$-free solution. Comparison of the two records at pH 7.4 shows that the negative after-potential is somewhat diminished and the maximum of the positive potential comes somewhat earlier, but the differences are not striking. At pH 8.0, on the other hand, the rhythmicity and the amount of discharge set the pictures taken in the two states into marked contrast. The pictures differ in much the same way that the Spring series and the October series previously mentioned differed, suggesting that the condition of the calcium was not the same at the two times of the year.

The effect of exclusion of KCl from the Krebs solution was, as might be expected, the opposite of that produced by a $CaCl_2$-free solution. The effects produced after exposure to the KCl-free solution for eight hours are shown in the third column of figure 2 and represent a continuation of the experimentation on the nerve, the behavior of which is depicted in the other parts of the figure. The thresholds were raised and no spontaneous discharge occurred when the nerve was made alkaline. The negative after-potential was increased at all reactions and the positive after-potential decreased, in comparison with the pictures obtained in the solution of normal composition at the same pH (column 1). At pH 7.4 the positive

after-potential disappeared altogether and its size at pH 8.0 was comparable to that normally obtaining at pH 7.4; in fact, the KCl-deprived nerve gave a response of almost normal form at pH 8.0.

The effect of sodium citrate. Deionization of the calcium by addition of citrate to a complete solution proved an effective way of bringing out the same changes in the nerve that had been brought out by other methods of decreasing the number of calcium ions or the Ca^{++}/K^{+} ratio. An amount of isotonic sodium citrate sufficient to deionize all the calcium (3 per cent—see Hirschlaff, 1935–36) was added to the stock solution and the nerve

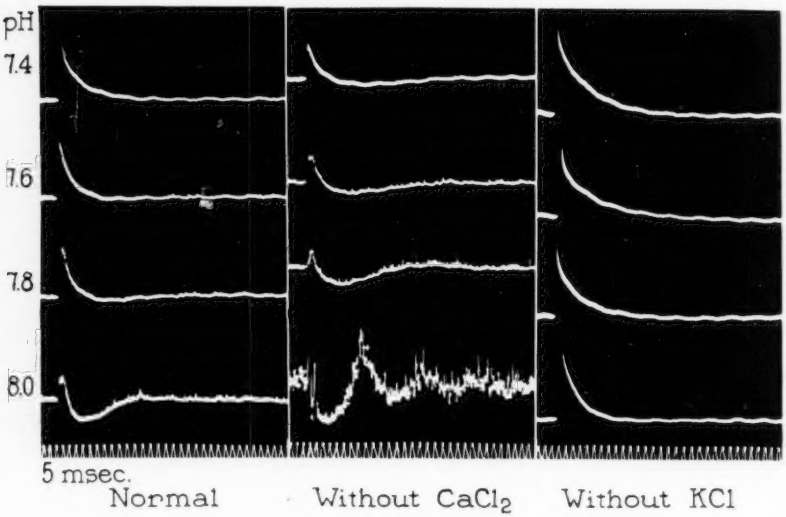

Fig. 2. First column: pH changes in the after-potentials (phrenic nerve) at normal composition of Krebs solution. Second column: pH changes in the same nerve after 1 hour's soaking in calcium-free Krebs solution. Third column: pH changes after 8 hours' exposure to potassium-free Krebs solution following the exposure to the calcium-free solution. Temp. 37.5°C. Conduction distance, 4.5 cm. Lead distance, 2.0 cm.

exposed to the mixture for 80 minutes. The pictures obtained over the usual pH range are shown beside the corresponding control pictures in figure 3. The difference between the two series is most striking at pH 7.8.

Discussion. In general, the effects of calcium and potassium ions upon the responses of mammalian nerve have proven to be the same as upon frog nerve. H. T. Graham (1933) found that excess calcium increases the negative after-potential in frog nerve and that excess potassium decreases it. The same is true for mammalian nerve, if the statement is restricted to the first negative after-potential,—for dominance of potassium (de-

crease of calcium) is responsible for an increase of negative after-potential in the period following the positive after-potential. When the after-potential is rhythmic this increase appears in the negative phase of the cycles. In addition, dominance of potassium increases the positive potential (a point which has not been examined in frog nerve), shortens its duration, and when the nerve is rhythmic, shortens the period of the rhythm.

As in frog nerve, the effect of the ions upon the after-potential is differential with respect to the spike, large effects in the after-potentials being

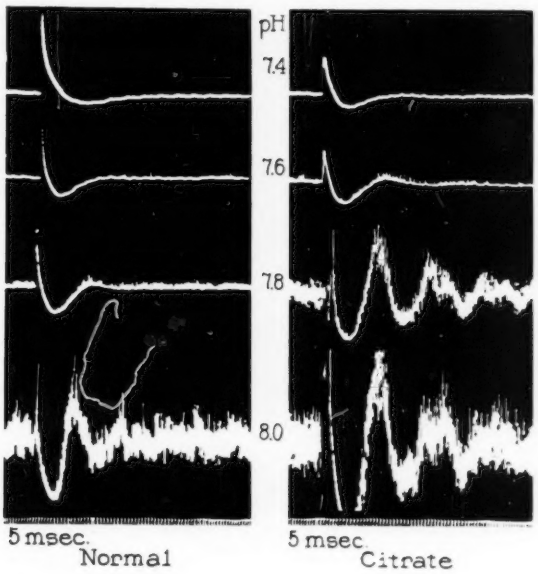

Fig. 3. First column: pH changes in the after-potentials (phrenic nerve) by normal composition of the Krebs solution. Second column: pH changes in the same nerve after 80 minutes' exposure to the same saline solution, calcium being deionized by sodium citrate. Temp. 37°C. Conduction distance, 4.5 cm. Lead distance, 2.0 cm.

accompanied by little change in the spike magnitude. According to H. T. Graham's observations on frog nerve, the tendency for the spike height to decrease is greater under the influence of potassium than it is under the influence of calcium. This point has not been made a subject for special study in this paper but such observations as have been made are in accord with the finding in frog nerve.

The point of major importance which demands discussion is the relation between the effects produced by hydrogen ions and those produced by

calcium ions—or, inversely stated, those produced by potassium ions and by hydroxyl ions. Whether the change of threshold is under consideration, or the alteration in contour of the after-potentials or the amount of spontaneous discharge, the statement holds that all the effects which are produced by shifts in the pH of the nerve can be reproduced by an alteration of the ion balance. In this regard these functions are in accord with other instances—both physiological and histological—in which resemblances have been shown between the action of Ca^{++} and H^+, on the one hand, and of K^+ and OH^- on the other (Bethe, 1920; Höber, 1926–27; Rosenberg and Kitayama, 1929–30; Graham, 1933).

Therefore, inasmuch as the pH of the solutions would determine the degree of ionization of the calcium, the question might be asked whether the effects produced by shifts in pH might not all be attributable to the state of the calcium. A definite answer to this question can not be given. On the one hand, the data concerning the electrolytes and proteins which would be necessary for calculation of the ionization of the calcium in terms of pH according to the formula of Hastings and his co-workers (1934) are lacking, and on the other hand, the experiments do not supply information about the true activity of the calcium in the nerve fibers which corresponds to the states observed under the action of the several solutions unbalanced as to calcium.

Whatever the definite answer may be, it must be admitted that the ionization of the calcium is an important factor in determining the changes produced by alteration of the hydrogen ion concentration and also that the effect produced by any given Ca^{++}/K^+ ratio varies greatly, depending upon the reaction of the solution in which it is operating. The latter fact provides a procedure of practical importance for detecting an abnormal ion balance. A small imbalance may produce but a small deviation from normal at pH 7.4, but a large deviation if the nerve is forced to operate at a reaction unfavorable to optimal activity of the salts which are present. In the experiment recorded in figure 2 it will be recalled that while the abnormality was small at pH 7.4 it was marked at pH 7.8. In other words, the most satisfactory method for detecting an improper ion balance is to examine the nature of the nerve's response to stimulation at reactions other than normal.

SUMMARY

The effect of physiological salines of different composition was observed on single responses of mammalian nerve and the results were compared with those arising from alteration of the hydrogen ion concentration.

Exclusion of calcium chloride from the solution produced at pH 7.4 the same changes which were produced by a high pH when the composition of the solution was normal. The threshold to electrical excitation was

lowered, spontaneous discharges occurred and the after-potentials changed in a characteristic way. The early negative portion was decreased, the positive portion increased, and rhythmicity appeared.

Deionization of the calcium with citrate produced similar effects, and exclusion of potassium from the solution caused changes in the opposite direction. The latter resembled those occurring when the acidity of the nerve is increased.

The alterations of the nerve attendant upon variations in the salt content of the solutions were very slowly established.

Recognition of small deviations from a proper ionic balance can often best be accomplished by examining the nerve in solutions the pH value of which is above normal.

The similarity of the effects produced by deionization of the calcium, or the removal of the latter from the nerve, to those produced by increasing the pH supports the conclusion that the effect caused by an increased pH is mainly attributable to deionization of the calcium. Analogously, the effects produced by a decreased pH would be attributable mainly to ionization of the calcium.

I wish to express my sincere gratitude to Dr. Herbert S. Gasser for his advice and encouragement during this investigation.

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CORTICOADRENAL INSUFFICIENCY: METABOLISM STUDIES ON POTASSIUM, SODIUM AND CHLORIDE

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The results of many investigations (1, 2, 3, 4, 5, 6, 7) have now shown that the adrenal cortex is concerned with the regulation of sodium, potassium and chloride in the animal organism. Since it was found that adrenalectomized dogs under optimal environmental conditions could be maintained indefinitely if sufficient sodium chloride and sodium citrate or sodium bicarbonate were given, a further study was undertaken to determine quantitatively the metabolic balance and the concentration of these substances in the blood serum and cells of animals that were maintained with the salts alone without the use of any adrenal cortical extract. Furthermore, it was found desirable to establish quantitative relations in regard to the distribution of potassium in the cells and serum when acute symptoms of adrenal insufficiency were produced in adrenalectomized dogs by the oral administration of potassium salts. This paper therefore presents the results of a study of the distribution of these constituents in adrenalectomized dogs compared to that in an intact dog, with some observations on the blood urea, alkaline reserve and blood sugar.

CARE OF ANIMALS AND METHODS. One intact female control dog and three adrenalectomized male dogs of proved sensitivity (dogs A, B and C), were kept on a low potassium diet made up of commercial casein, 25 per cent; lard, 18 per cent; butter, 7 per cent; sucrose, 25 per cent; cracker meal, 22 per cent; dried brewer's yeast, 2 per cent, and bone ash, 1 per cent, by weight. This was fed at a level of about 150 per cent of basal requirements for calories. In addition the dogs received 15 grams of sodium chloride, 5 grams of sodium citrate and 0.5 gram of supple-

[1] National Research Council Fellow in the Biological Sciences at The Mayo Foundation. I wish to express my deep appreciation to Dr. E. C. Kendall, Head of the Section on Biochemistry, for suggesting the general problem and for so actively coöperating in providing facilities for conducting these experiments. Sincere thanks are also due Miss E. R. Hartzler, M.S., for extensive aid in the chemical phases of these studies, and in particular Dr. F. C. Mann, Head of the Institute for Experimental Medicine, for providing the adrenalectomized dogs and facilities for their care.

[2] Now residing in College Park, Maryland.

mentary salt mixture (magnesium citrate, 55 per cent; ferric citrate, 27 per cent; manganese sulphate, 6 per cent, and copper sulphate, 12 per cent, by weight).

In these studies about 12 cc. of blood was taken from the jugular vein and defibrinated by stirring; 0.5 cc. was used for sugar analysis (by Folin's method, 1929), 2 cc. was used for the determination of urea (by Van Slyke's aeration method) and the remainder was centrifuged in hematocrit tubes. The serum and cells were separated and duplicate microdeterminations, using trichloracetic acid filtrate, were made for potassium (by Sobel and Kramer's method, 1933), and for sodium (by Weinback's method, 1935). One-half cubic centimeter of serum was used for the determination of the carbon dioxide combining power (by Van Slyke and Cullen's method, 1917). For the metabolic data, the sodium was determined by the macro zinc uranyl-acetate method, the potassium by the macro chloroplatinate method and the chloride by Wilson and Ball's method (1928).

Maintenance studies. Apparently the adrenalectomized dog on this diet is fully able to maintain the constituents of the blood within ranges which appear to be normal (table 1). However, the daily metabolic data (figs. 1, 2 and 3) show that the adrenalectomized dogs under optimal conditions required much greater positive balances of sodium and chloride than the intact dog, and that they had less control over the output of these substances in the urine. The fecal output was insignificant in every case. The instability in regard to the control of the elimination of sodium and chloride probably indicates why the adrenalectomized animals are so sensitive to environmental stress.

Inspection of data and the simple correlation coefficients in table 2 indicate that the values for urea, sugar and potassium in the blood show no significant correlations with those of sodium and chloride. This is undoubtedly a result of the constant, high intake of sodium chloride and the low intake of potassium during the maintenance period. There was a definite positive correlation between the sodium in the serum and in the cells; on the other hand there was a negative correlation between the sodium in the serum and in the cells and the chloride in the cells. The chloride in the serum was generally without prediction value.

Studies on the toxicity of potassium. Allers and Kendall (2) have shown that the syndrome of adrenal insufficiency can be precipitated by the addition of small amounts of potassium to the diet, but their protocols indicate that the sensitivity of the dogs varied considerably, depending on individual resistance, the condition of the animal, and the level of potassium fed. The animals used in the present tests were all in excellent condition and the potassium content of the blood indicated that a stabilized level had been reached. Potassium dihydrogen phosphate was fed

in an initial daily amount of 0.5 gram, and this was increased daily by increments of 0.5 gram of potassium until the crisis of adrenal insufficiency was precipitated. This was a necessary procedure because previous work in this laboratory had indicated that dogs fed uniform subtoxic doses of potassium could adjust themselves to it within a few days and then would appear normal until the dose of potassium was again increased. The food containing the potassium salt generally had to be force-fed, since the adrenalectomized dogs refused to eat these diets after the first

TABLE 1

Weekly or biweekly studies of the blood of dogs maintained on a low potassium diet

NUMBER OF SAMPLES OF BLOOD	INTACT DOG 29		ADRENALECTOMIZED DOGS 45		DIFFERENCE OF MEANS
	Mean	C.V.*	Mean†	C.V.	
Blood urea, mgm./100 cc.	20	20	34	23	14‡
Blood sugar, mgm./100 cc.	54	18	56	14	2
Cell volume, per cent	48	7	31	11	17‡
Serum:					
Potassium, mgm./100 cc.	16	21	17	15	1
Sodium, mgm./100 cc.	330	3	325	3	5
Chloride, mgm./100 cc.	411	4	407	3	4
CO_2 combining power, volume per cent	34	10	40	12	6‡
Cells:					
Potassium, mgm./100 cc.	25	16	26	18	1
Sodium, mgm./100 cc.	248	4	248	5	0
Chloride, mgm./100 cc.	213	8	213	8	0

* Coefficient of variation in per cent.

† The adrenalectomized dog B varied significantly (9 determinations) in cell chloride, +13 mgm. per 100 cc.

The adrenalectomized dog C varied significantly from the joint mean (4 determinations) in blood sugar, −13, and cell volume, +6 mgm. per 100 cc.

‡ Statistically significant (at least 2.5 times S.E.D.).

day. The intact dog relished its food, however, even though it contained as much as 35 grams of potassium dihydrogen phosphate daily.

The intact dog (figs. 1 and 4) appeared to be in distress during the first two days following the initial feeding of potassium. The concentration of potassium in the cells rose precipitously about 12 mgm. per 100 cc. during these two days, but on the third day again returned to normal. The first major effect of an increased intake of potassium in this case was on the concentration of potassium in the cells. The animal continued to store potassium, but this storage must have taken place in tissues where

the potassium could not significantly affect the animal's health. The small positive balance of sodium and chloride was not affected until the dog was fed 10 grams of potassium on two successive days. The dog still appeared normal, even at this high intake of potassium. The following day no additional potassium was given and equilibrium was again established.

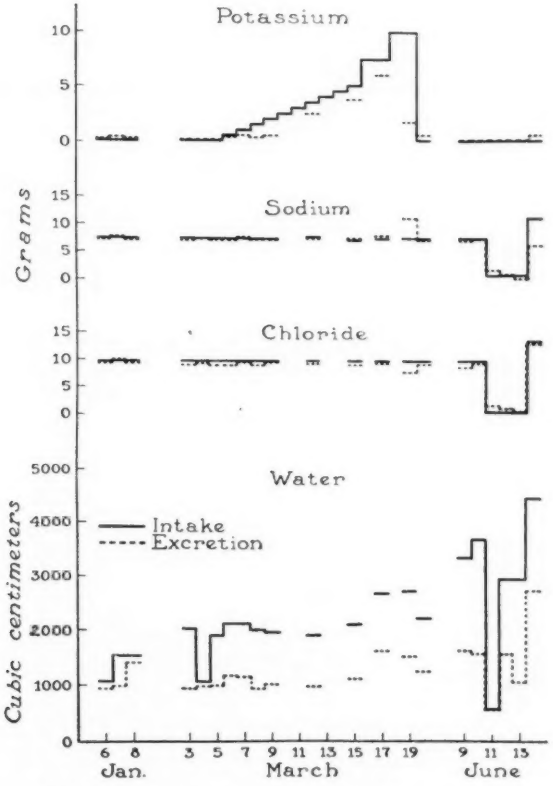

Fig. 1. Daily balance metabolic data for control dog

In the case of the adrenalectomized dog A (fig. 2) whose second adrenal gland was removed June 20, 1935, the feeding of potassium promoted diuresis which brought about some elimination of potassium, but this also had an adverse effect on the sodium and chloride balance. The level of potassium (fig. 5) rose quickly in the cells and progressively in the serum until a crisis was precipitated. There was a marked increase in the

hematocrit value and a decrease in the concentration of sodium in the cells. However, there was no marked increase in the concentration of urea, which has generally been associated with crisis.

The dog recovered from crisis when given a single intravenous injection of 30 grams of glucose, 7 grams of sodium chloride and 3 grams of sodium

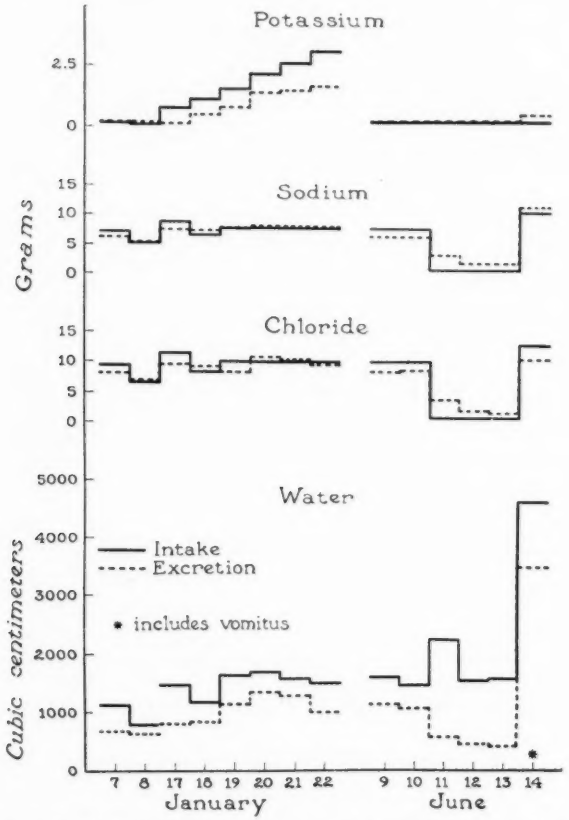

Fig. 2. Daily balance metabolic data of adrenalectomized dog A

bicarbonate in 200 cc. of water, and with the administration of large amounts of adrenal cortical extract for twenty-four hours. Two days following the crisis the constituents of the blood were being maintained at approximately normal levels. Incidentally, the high value for cell potassium (40 mgm. per 100 cc.) on January 9 resulted from a state of insufficiency precipitated on January 7 (after fifty-eight days on the low

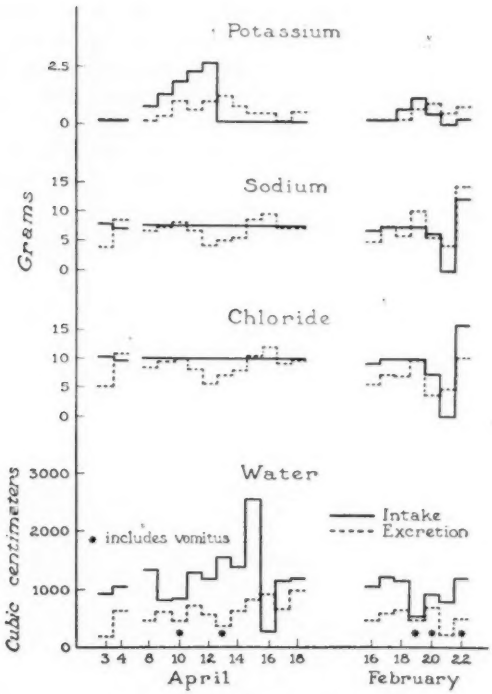

Fig. 3. Daily balance metabolic data for adrenalectomized dog C during April (left), and for dog B during February (righ

TABLE 2

Simple correlation coefficients of constituents of blood of dogs maintained on a low potassium diet

NUMBER OF COMPARISONS	INTACT DOG 29	ADRENALECTOMIZED DOG	
		A	B
		32	9
r Na s—Na c*	0.46†	0.50†	0.71†
r Na s—Cl s	−0.22	0.06	0.29
r Na s—Cl c	−0.42†	−0.51†	−0.29
r Na c—Cl s	−0.37	0.18	0.41
r Na c—Cl c	−0.41†	−0.20	−0.70†
r Cl s—Cl c	0.43†	0.10	−0.45
r K s—Na s	0.18	0.14	
r Na intake—Na s		0.19	

* s = serum; c = cells.

† Statistically significant.

potassium diet) by the animal's continued refusal to eat all of its food. The dog recovered, however, when it voluntarily consumed all of its daily ration. In this instance the serum potassium apparently did not reach a critical level in the presence of the high concentration of sodium and chloride.

Dog B (figs. 3 and 6) whose second adrenal gland was removed October 29, 1935, proved to be very sensitive to potassium, which in this case did

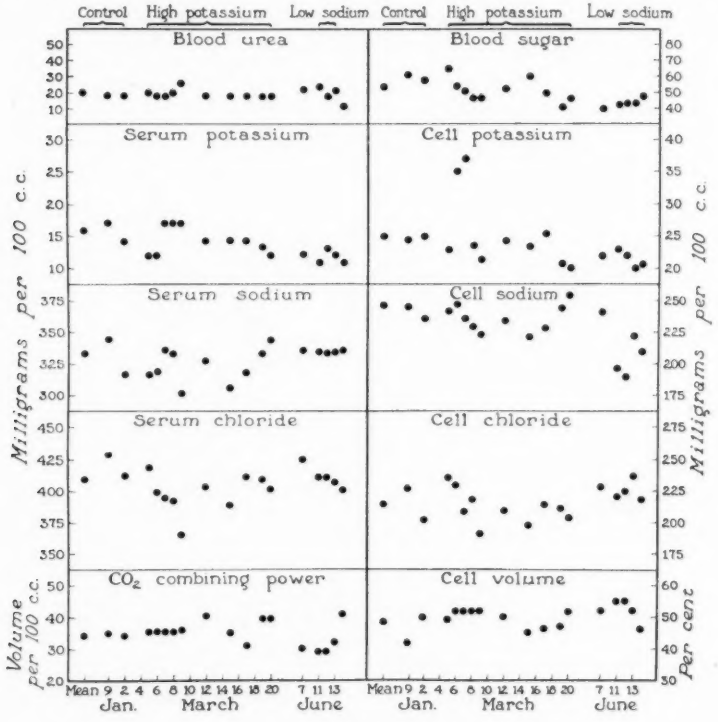

Fig. 4. Hematologic data for control dog

not promote diuresis but did cause nausea and vomiting. The concentration of potassium in the cells rose rather quickly from a high normal to a critical level without a marked increase in the level of potassium in the serum. There was also a rather marked decrease in the concentration of chloride in the serum and cells. This dog did not recover as quickly as dog A and needed repeated administrations of sodium salts and adrenal cortical extract. The mineral constituents of the blood were approximately normal four days following crisis and the animal's general appearance was much improved.

In the case of dog C (figs. 3 and 7), whose second adrenal gland was removed October 29, 1935, the administration of potassium in the diet also promoted mild diuresis and caused nausea and vomiting. The

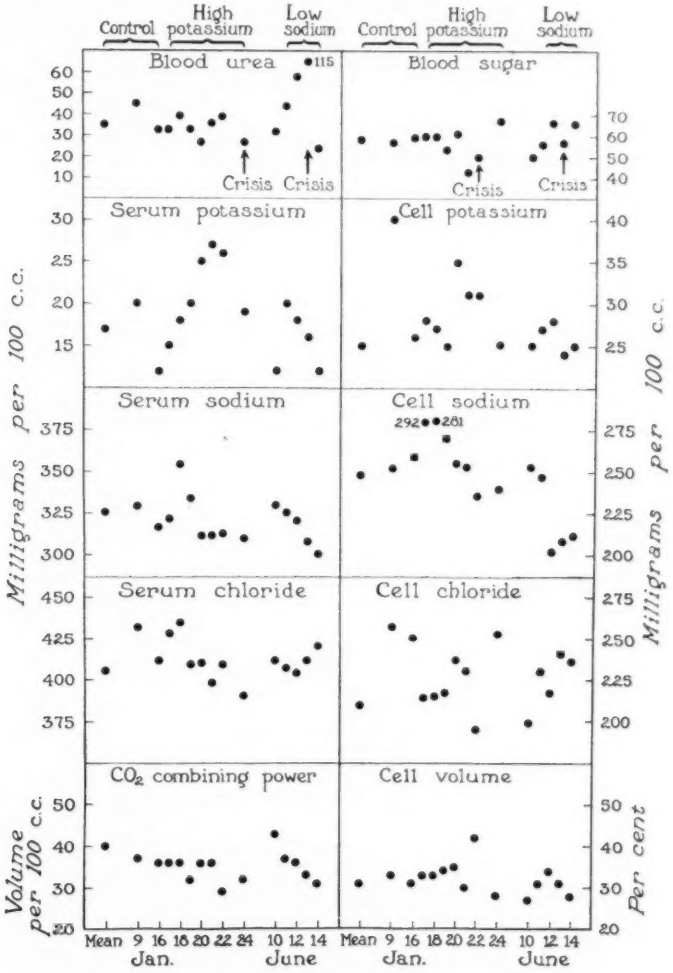

Fig. 5. Hematologic data for adrenalectomized dog A

hematologic data reveal the characteristic condition, except for the rather high level of urea at the time of crisis. The level of urea in the blood is probably of secondary importance, because adrenalectomized dogs have

been in severe crisis with a value for blood urea of 39 mgm. per 100 cc. and have lived in reasonably good health with a level of urea of 150 mgm. per 100 cc. for a week at a time.

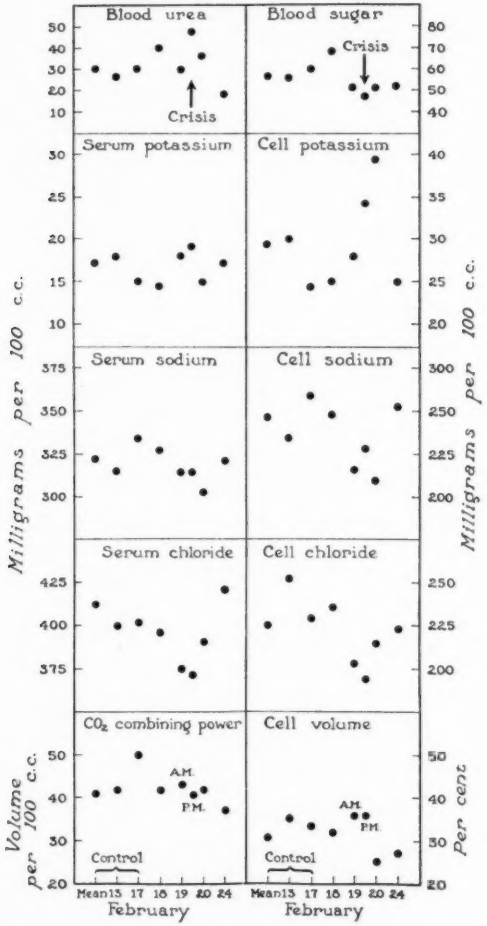

Fig. 6. Hematologic data for adrenalectomized dog B

Although dog C showed definite signs of slipping into severe insufficiency, the final stage was not pressed by increasing the intake of potassium; instead, the low potassium, high sodium chloride-sodium citrate diet was force fed and the dog recovered. On April 17, when the dog

again appeared to be normal, a large dose of adrenal cortical extract was given. Apparently the dog had been brought back to normal solely by restriction of the intake of potassium, since the administration of adrenal

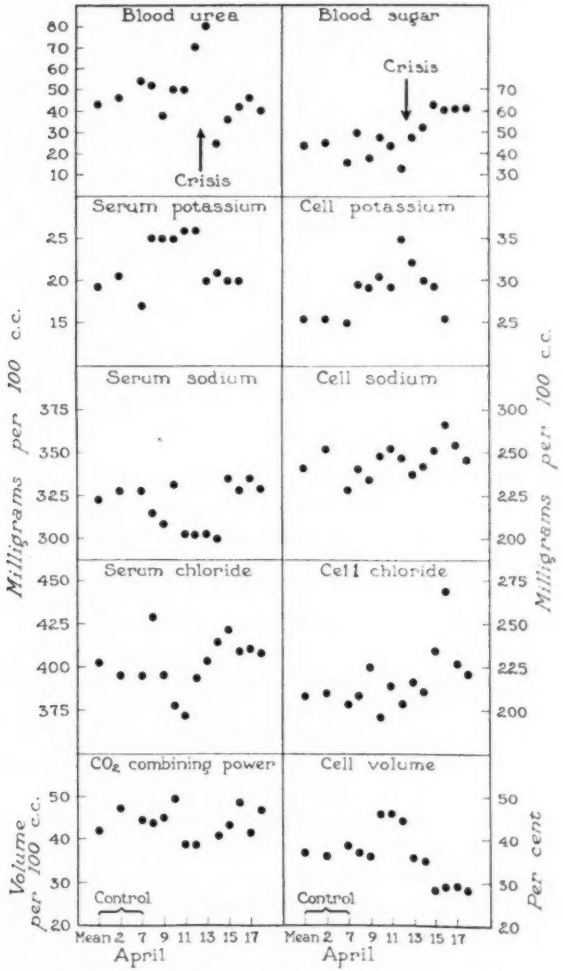

Fig. 7. Hematologic data for adrenalectomized dog C

cortical extract produced only little change in the elimination of mineral constituents or their concentration in the blood.

The effect of low sodium content of the diet. The final test was to deter-

mine the sensitivity of the intact dog and dog A to the same diet which was given without the added sodium salts. The long period on the low potassium, high sodium chloride-sodium citrate diet had decreased the level of potassium in the serum, but did not produce discernible symptoms of potassium deficiency. The data in figures 1, 2, 4 and 5 indicate that the intact dog was able to readjust itself quickly to the low salt intake without any marked changes in the composition of the blood, but that the adrenalectomized dog A lost considerable sodium and chloride, although there was a diminution in the output of urine. There was a progressive loss of sodium from the serum and cells without affecting the levels for chloride, and there was a rise in the serum and cell potassium.

Dog A passed into severe crisis after only three days on the low sodium diet. It was necessary to give intravenous injections of glucose, sodium chloride and sodium bicarbonate and very large quantities of adrenal cortical extract to save the animal. The dog was practically prostrate and had a decreased temperature and depressed heart beat, and it could scarcely drink water without vomiting. The intact female was given the same treatment for control purposes, but only insignificant changes were noted in the levels of the constituents of the blood and there was no apparent effect on the animal's health.

Comment. Since fatal insufficiency is characterized by cardiac failure, it is possible that insufficiency is promoted by an uncontrolled ion antagonism in the adrenalectomized dog which is not protected by a high intake of sodium and chloride or adrenal cortical hormone. It is quite conceivable that the cortical hormone is concerned with the rate of exchange of potassium through the cell membrane. This may explain the high level of potassium in the cells of the intact dog for the first two days of potassium feeding (fig. 4). Possibly the mechanism controlled by the adrenal gland was not functioning adequately, because the dog had been on the low potassium, high sodium chloride-sodium citrate diet for 115 days prior to this test, and a few days of stress (high intake of potassium) were necessary to activate the glands to their normal function.

An interesting and possibly significant finding in these studies is that the concentration of potassium in the cells increased when crisis was imminent. The data indicate that, under the conditions of these experiments, the cell membranes are affected, as it seems improbable that the introduction of newly formed cells with a distinctly different composition could account for the differences noted. This is especially true of the marked changes in the concentration of potassium that take place within twenty-four hours during recovery after a severe crisis.

Adrenalectomized dogs cannot rigidly husband the sodium and chloride given, and, in the absence of a high intake of sodium salts, cannot control the distribution of potassium without the aid of the adrenal cortical hormone.

SUMMARY AND CONCLUSIONS

Adrenalectomized dogs may be maintained satisfactorily without adrenal cortical extract on a diet which has a low content of potassium and a high content of sodium chloride and sodium citrate. Such dogs require a much greater positive balance of sodium and chloride than do intact animals, and they are less able to maintain a uniform daily balance.

Symptoms of acute adrenal insufficiency caused by a high intake of potassium are associated with a high level of potassium in the cells. Recovery, produced either by the administration of sodium salts or by injection of adrenal cortical extract, or both, is accompanied by a return to the normal levels of potassium in the blood. The symptoms of adrenal insufficiency may or may not be accompanied by a high level of blood urea. There are changes in the concentration of blood sugar, sodium, chloride, and hematocrit value, but only the increase in potassium was found to be characteristic. Symptoms of adrenal insufficiency due to a low intake of sodium and chloride simulate those produced by a high intake of potassium. The adrenal cortical hormone probably is concerned with the permeability of cell membranes.

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